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Investigating the Role of Spiders in Integrated Pest Management for Biological Control of Nebraska Crop Pests

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Investigating the Role of Spiders in Integrated Pest Management for Biological Control of Nebraska Crop Pests

By

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Investigating the Role of Spiders in Integrated Pest Management for Biological Control of Nebraska Crop Pests

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As generalist predators, spiders are capable of fulfilling a wide variety of ecological niches allowing them to intercept and prey upon an array of insect species. This characteristic could be particularly advantageous within agricultural ecosystems. This project sought to reveal the composition and abundance of spider communities within corn fields in western Nebraska and the impact of agronomic practices on those communities as well as determine the potential for spider predation upon two key pests of corn. Spiders were collected from eight corn fields from May to August 2017 and four corn fields from May to August 2018. Additionally, the populations of two important pests of corn, western corn rootworm (*Diabrotica virgifera virgifera*) and western bean cutworm (*Striacosta albicosta*), were sampled to determine prey availability. During the course of this study, a total of 1,011 spider specimens composed of 15 families, 34 genera and 53 species were collected from the 12 field sites for both years combined. Of the total specimen count, 300 (30%) were immatures. Two families, Lycosidae and Linyphiidae, made up 60% (n = 605) and 24% (n = 244) of all specimens collected, respectively. Gnaphosidae (n = 36) and Thomisidae (n=36) were the third most common families collected, representing 4% each of all spiders collected. The impact of three agronomic practices (tillage, crop rotation and insecticide use) on spider species diversity and evenness was evaluated. Species evenness was not significantly affected by any of the practices while species diversity was significantly impacted by insecticide use alone. Pest population sampling revealed

presence of both western corn rootworm and western bean cutworm in the sampled fields. Field-collected Thomisidae and Lycosidae were screened for target prey DNA: none of the screened thomisids tested positive for *S. albicosta* DNA while only two lycosids tested positive for *D. v. virgifera* DNA. While these results indicate no to very low predation of these pests by the selected spider families in the field, a variety of factors including short DNA detectability windows, spider feeding habits and prey availability at the time of spider capture may help to explain these results and illustrate a need for additional studies. These results reveal the abundance and composition of spider communities in Nebraska corn fields as well as the role of spiders within agroecosystems. The results can be utilized to develop improved conservation biological control programs in the future.

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CHAPTER 1: Literature Review

Introduction

This review seeks to summarize existing data regarding spider biology and ecology, biological control of pest insects by spiders within annual and perennial agroecosystems and the biology, behavior and control methods of two key pests of corn in Nebraska.

As generalist predators, spiders are capable of fulfilling a wide variety of ecological niches allowing them to intercept and consume an array of insect species. This characteristic can be particularly advantageous for biological control within agricultural ecosystems. Within such ecosystems, the spider community can be described in terms of guilds which distinguish the predatory behavior and potential prey of spiders. Spider communities comprising several different guilds are generally more likely to suppress insect pests than more homogenous communities. Studies exploring the biological control potential of spiders have been conducted in annual cropping systems, such as corn, cotton, soybean, and wheat, as well as in perennial systems like alfalfa and orchards. The data acquired from these studies has shown that spiders can be beneficial and contribute to pest insect suppression. By taking the spider community into consideration when choosing field management protocols, producers will benefit from the various ecological services that a diverse spider population can provide while simultaneously enhancing the biodiversity and stability within their fields.

A variety of pest insect species exist in Nebraska with the western corn rootworm (*Diabrotica virgifera virgifera*) and ear feeding caterpillars, like western bean cutworm (*Striacosta albicosta*), being the primary economic pests of corn. It has been shown that

spiders are co-inhabiting agroecosystems with western corn rootworm and western bean cutworm (Oliveira-Hofman et al. 2018) and that cursorial spiders prey on *D. v. virgifera* larvae (Lundgren et al. 2009); however, a study has yet to be done to determine what trophic relationships might exist between spiders of various guilds and these two pests.

Spider Biology & Ecology

Spiders are non-insect arthropods belonging to the taxonomic class Arachnida and order Araneae. Currently, there are more than 48,000 known species of spiders worldwide belonging to 120 families (World Spider Catalog, 2020). The vast majority of spiders are predatory; however, some species are known to supplement their diet with plant material, including nectar and pollen (Meehan et al., 2009; Peterson et al., 2016; Taylor & Bradley, 2009). Most spider species are generalist predators, although prey specialization does occasionally occur (Nentwig, 1986; Pekár & Toft, 2015). As generalist predators, spiders play an integral role within a wide variety of ecosystems. They inhabit every continent except Antarctica and can be found in some of the most extreme environments on Earth including alpine areas reaching altitudes above 4000 meters (Schmoller, 1970), the high Arctic (Ernst et al., 2016; Sikes et al., 2013), the Southern Ocean Islands near Antarctica (Pugh, 2004), within freshwater (Seymour & Hetz, 2011; Bleckmann & Lotz, 1987), as well as the salt flats and deserts of the southwestern U.S. (Crews & Gillespie, 2014; Kay et al., 1970). Their evolution has led to a wide range of morphological and behavioral differences that allow multiple species to coexist within an ecosystem (Uetz, 1977; Dimitrov & Hormiga, 2020). This coexistence can be attributed to the vertical spatial distribution of spiders in which a variety of species will inhabit different niches throughout the same habitat (Waldorf, 1976; Benhadi-Marín

et al., 2020). Some species build webs in order to trap and kill prey while others actively seek out or ambush their prey. These and other diverse hunting strategies among spiders gives them the ability to attack and kill a variety of insects, making them efficient predators.

The ubiquity of spiders can be attributed, in part, to their hardiness and ability to survive in less than favorable conditions. Because they feed on a variety of prey, spiders have the ability to survive even when more desirable prey is unavailable. Furthermore, spiders have a low metabolic rate that, in some instances, can be further reduced by 30 – 40% during periods of starvation resulting from prey scarcity (Anderson, 1974). Scarcity of prey is not the only challenge spiders must contend with. Species inhabiting temperate climates must adapt to significant temperature fluctuations throughout the year. As an example, species belonging to the families Philodromidae and Clubionidae have adapted to sub-zero winter temperatures by reducing the supercooling point of their hemolymph through the production of specialized proteins and glycerol (Duman, 1979). Spiders must also regulate their internal temperature during the summer and in hot climates. For example, a species of orb-web spider, *Argiope trifasciata* (Forsskål), will change the orientation of its web to the sun to regulate its body temperature and avoid overheating (Tolbert, 1979). These examples highlight only a few of the adaptations spiders have evolved that contribute to their ubiquity in less than favorable environments.

The nearly worldwide distribution of spiders can also be attributed to another important characteristic: their ability to colonize new habitats. Spiders are capable of dispersing between habitats by two methods: aerial and cursorial (Ehmann, 1994). Aerial dispersal involves the utilization of strands of silk to float on air currents, a process

known as “ballooning” (Foelix, 2011). Ballooning spiders have been collected by airplane as high as 5 kilometers (Glick, 1939) and can travel hundreds of kilometers horizontally (Okuma & Kisimoto, 1981). This aeronautic behavior can be found among several spider families; however, it most often occurs in juveniles and small adults (Ehmann, 1994; Richter, 1970). Cursorial dispersal, or moving along the ground, is generally more localized and does not involve the long distances of aerial dispersal. For example, Linyphiidae, small spiders known to utilize ballooning, have been found in relatively equal numbers within corn fields bordering forest habitat, while the primarily cursorial Lycosidae exhibited a decrease in abundance as distance from the forest increased (Royauté & Buddle, 2012). Additionally, significant differences in aerial dispersal abilities have been shown in species belonging to the same genus (Richter, 1970). This suggests that the dispersal ability of a spider community may be dependent on the species composition of that community. Even so, spiders are incredibly adept at colonizing new habitats, even those that have been disturbed. In temperate zone corn fields, for example, significant numbers of spiders have been found to be active immediately following snow melt in early spring (Royauté & Buddle, 2012). Even habitats that have been destroyed by natural disaster, such as volcanic eruption, are rapidly colonized by spiders (Crawford et. al., 1995). The ability of spiders to colonize such habitats before many other organisms increases their potential value in agroecosystems which are often disturbed (from harvest, tillage, etc.) on a regular basis.

Within the ecosystems they inhabit, spiders play a vital role as predators, prey for other arthropods and vertebrates, and even contributors to decomposition. Although the vast majority of spiders are quite small, their impact on the insect population is quite

significant. It has been estimated that the world spider population kills 400 – 800 million metric tons of prey per year and over 90% of that prey is insects (Nyffeler & Birkhofer, 2017). The majority of these insect prey belong to seven orders: Diptera (flies), Hemiptera (true bugs, aphids), Hymenoptera (bees, ants), Collembola (springtails), Coleoptera (beetles), Lepidoptera (butterflies and moths), and Orthoptera (grasshoppers and crickets) (Nyffeler & Birkhofer, 2017). This is potentially significant since many insect pests of crops belong to the orders Lepidoptera, Hemiptera, and Coleoptera. In addition to their role as predators, spiders are also an important source of food for a variety of other arthropods as well as vertebrates. Predatory insects as well as parasitoid wasps and flies often specialize on spiders (Horton, 1983). Vertebrate predators such as birds (Horton, 1983; Gunnarsson, 2007), small reptiles and rodents (Wise and Chen, 1999) rely on spiders as a source of nutrition. This further illustrates the important role spiders play within the food webs of ecosystems. Spiders also contribute to ecosystem health in a few unexpected ways. They have been shown to impact the decomposition of forest floor leaf litter. When spiders were removed from test plots, decomposition was reduced by as much as 20% (Lawrence and Wise, 2004). This is important to consider since litter decomposition has a direct effect on nutrient-cycling and soil nitrogen content (Bonanomi et al., 2017). There is also some evidence that the presence of spiders and their excrement may interact in a beneficial way with soil microbes (Smith and Wilder, 2019). Potted plants that contained spiders had increased biomass and produced a greater number of pods and flowers than those plants in containers without spiders (Smith and Wilder, 2019). These studies indicate that spiders may play a much wider role in ecosystem health than previously thought.

Spiders in Agroecosystems

While cropping systems are considered a type of ecosystem, broadly known as agroecosystems, they have a variety of unique characteristics that do not exist in natural, unmanaged systems. When comparing the community structure of spiders, there is often a trend of higher diversity in natural systems versus managed systems while abundance is often not significantly different between the two ecosystem types (Roughley et al., 2006; Muma & Muma, 1949; Kerzicnik et al., 2013). This reduction in diversity can be attributed to two unique characteristics of cropping systems: habitat homogeneity and management practices. Agroecosystems often consist of monocultures that do not provide the habitat heterogeneity necessary to support a biodiverse fauna (Benton et al., 2003). Additionally, the various management practices and inputs applied to agroecosystems can be detrimental to potentially beneficial predatory arthropods (Thorbeck & Bilde, 2004). Spiders in particular have been shown to be more vulnerable to mechanical management practices, such as mowing and tillage, than other predatory arthropods (Thorbeck & Bilde, 2004). Due to these factors, the nature of agroecosystems suppresses rather than supports spider diversity (Prieto-Benítez & Méndez, 2011). Despite this, a number of population surveys have shown that the majority of annual and perennial agroecosystems can support robust spider communities and therefore have the potential to benefit from the ecological services spiders provide (Nyffeler & Sunderland, 2003).

When attempting to describe the community structure of spider assemblages in agroecosystems, simply listing total numbers of families, genera, and species does not provide the information in a format relevant to biological control. For example, published data shows that in nine types of field crops within the United States, roughly 600 species

of spiders have been collected belonging to 192 genera and 26 families (Young and Edwards, 1990). While this provides a general overview of spiders in agroecosystems, it is necessary to consider crop types individually and to determine the foraging strategies and habitat use of spiders to understand their potential impact on various insect crop pests. This can be done by grouping spiders that utilize the same resources within a habitat into ecological guilds (Jaksic, 1981). While there has been inconsistency in the number and type of guilds spiders can be assigned to, from 11 guilds in Neotropical habitats (Dias et al., 2009) to only two guilds based solely on foraging strategy (Young and Edwards, 1990), one review has proposed eight guilds for spiders found specifically within crops (Uetz et al., 1999). For the purposes of the current review, this model will be used due to its relevance to agroecosystem spider communities. According to Uetz et al. (1999), spiders inhabiting agroecosystems can be grouped into one of eight guilds based on five ecological characteristics: foraging behavior, web type, use of microhabitat, level of mobility, and diel activity. Furthermore, the eight guilds can be grouped broadly into two main categories: hunting spiders and web-builders (Uetz et al., 1999). The hunting spiders include foliage runners, ground runners, stalkers, and ambushers while the web-building spiders are classified as sheet-web builders, wandering sheet/tangle weavers, orb weavers, and space web builders (Uetz et al., 1999). Each guild exhibits not only unique hunting strategies, but also tend to prefer different locations within a crop habitat (Table 1.1).

Table 1.1: A description of the spider guilds commonly found in crops (Uetz et al., 1999), including example spider families from each guild, the location(s) the guild is most commonly found in crops and hunting strategy.

Guild	Example Family (common name)	Location Found in Crops	Hunting Strategy
Ambusher	Thomisidae (crab spiders)	Hidden areas on foliage	Sit-and-wait predation
Stalker	Salticidae (jumping spiders)	Throughout canopy on exposed surfaces	Actively pursue and capture prey
Ground Runner	Lycosidae (wolf spiders)	Soil surface	Actively pursue or ambush prey
Foliage Runner	Philodromidae (running crab spiders)	Throughout canopy on exposed surfaces	Actively pursue and capture prey
Space-web Builder	Theridiidae (cobweb spiders)	Middle and top portions of foliage	Web capture
Orb-web Builder	Tetragnathidae (long-jawed orb weavers)	Middle portion of foliage; web sometimes spans area between plants	Web capture
Wandering Sheet-web Builder	Linyphiidae (dwarf spiders)	Middle portion of foliage and soil surface	Web capture
Sheet-web Builder	Agelenidae (funnel weavers)	Lower portion of foliage and soil surface	Web capture

Spiders belonging to the eight guilds have been collected from all major crops as summarized in the review of current literature in Table 1.2. The composition of spider guilds in each crop is illustrated in Figure 1.1. We can see from these data that spider

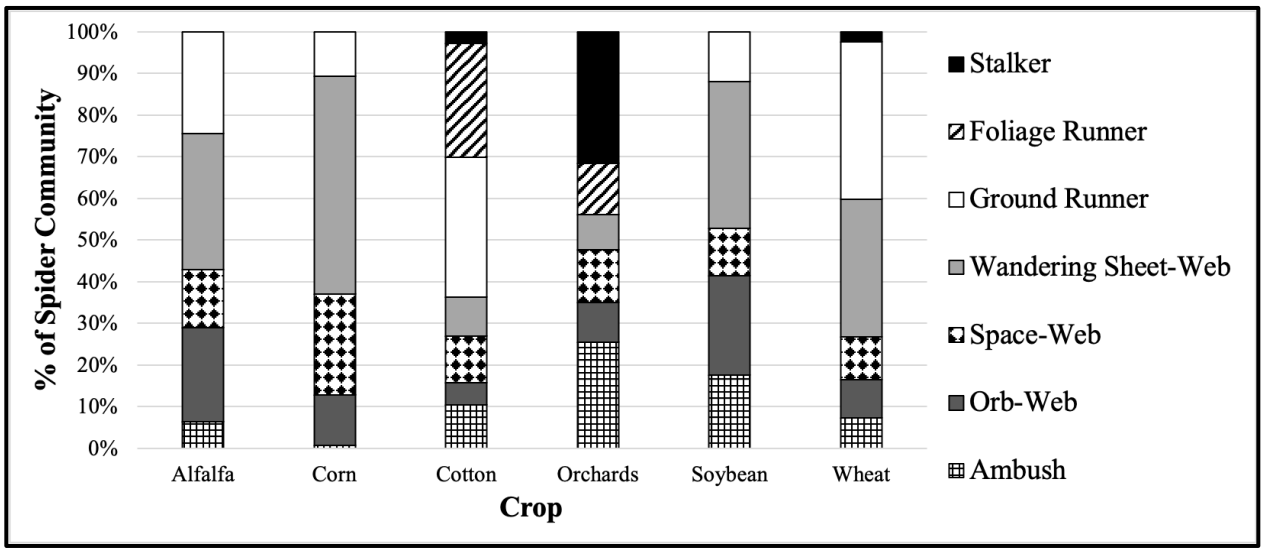
Table 1.2: A summary of selected studies evaluating the guild structure of spiders in major crops. Sampling method is included to account for biases towards ground-dwelling or foliage-dwelling guilds.

Study	Crop	Sampling Method(s)	Guilds Collected	Location of Study
Birkhofer et al. (2007)	**Alfalfa**	Hand	Wandering Sheet-Web, Orb-web	Kentucky, USA
Culin and Yeargan (1983)	**Alfalfa**	D-Vac; Pitfall	Orb-Web, Ambusher, Space-web	Kentucky, USA
Yeargan and Dondale (1974)	**Alfalfa**	D-Vac	Wandering Sheet-Web, Ground Runner, Space-web	California, USA

Ludy and Lang (2006)	**Corn**	Vacuum	Ambush, Wandering Sheet-web, Ground Runner, Ambusher, Space-web	Germany
Meissle and Lang (2005)	**Corn**	Various from plant	Ground Runner, Space-web, Ambusher	Germany
Schier (2006)	**Corn**	Pitfall	Wandering Sheet-Web, Orb-web	Germany
Volkmar et al. (2004)	**Corn**	Pitfall	Orb-Web, Ambusher	Germany
Dean and Sterling (1987)	**Cotton**	D-Vac	Wandering Sheet-Web, Ground Runner	Texas, USA
Dean et al. (1982)	**Cotton**	whole plant/pitfall	Orb-Web, Space-web, Ambusher, Foliage Runner, Ground Runner	Texas, USA
Mellet et al. (2006)	**Cotton**	Pitfall	Ground Runner, Space-web, Stalker, Wandering Sheet-web	South Africa
Bajwa and Aliniazee (2001)	**Orchards**	Beat sheet	Foliage Runner, Wandering Sheet-web, Ground Runner, Ambusher, Orb-web, Space-web	Oregon, USA
Dippenaar-Schoeman et al. (2005)	**Orchards**	Spray sheet	Stalker, Wandering Sheet-web, Foliage Runner, Ambusher, Space-web	South Africa
Specht & Dondale (1960)	**Orchards**	Beat sheet	Space-Web, Ambusher, Foliage Runner, Stalker	New Jersey, USA
Culin and Yeargan (1983)	**Soybean**	Pitfall, Shake-cloth	Ambusher, Orb-web, Stalker, Ambusher, Foliage Runner	Kentucky, USA
Kerzicnik et al. (2013)	**Wheat**	Pitfall, vacuum, hand	Space-Web, Ground Runner, Wandering sheet-web, Ambusher, Stalker	Colorado, USA

Pluess et al. (2010)	**Wheat**	Pitfall	Wandering Sheet-Web, Ground Runner, Space-web, Stalker	Israel
Seyfulina (2005)	**Wheat**	Pitfall, sweep-net	Wandering Sheet-Web, Ground Runner, Orb-web, Ambusher, Space-web	Russia

Figure 1.1: Data from studies listed in Table 1.2 illustrating the differences and similarities in spider guild structure between major crops.



communities vary in their guild structure between crop types, which is important when considering potential interactions between spiders and insect crop pests. The importance of the spider guild structure is evidenced by the vertical spatial distribution and wide variety of hunting strategies exhibited by spiders, which allows them to prey on insects of various types, sizes, and developmental stages. This might lend to their potential to provide ecological services, including biological control, within agroecosystems (Marc and Canard, 1997).

Spiders as Biological Control Agents

Biological control, or biocontrol, can be defined as the reduction of a pest organism's population by another living organism to a less damaging level (Eilenberg et al., 2001). Biocontrol is only one of several available management tools within an integrated pest management (IPM) program and is generally used to complement or enhance additional management approaches within an IPM framework. Currently, there are four main types of biocontrol: classical, inoculation, inundation, and conservation biological control (Eilenberg et al., 2001). The first three involve the intentional release of a biological control agent into a system in order to manage a pest below damaging levels (Flint, 2012). Conservation biological control focuses on supporting and enhancing beneficial organism populations that already exist within the system and is the type most applicable to spiders (Riechert, 1999).

Unlike other beneficial arthropods, such as parasitoids and specialist predators, there is currently little evidence that a single spider species is capable of reducing a pest population (Riechert & Lawrence, 1997). However, spiders can be successful in reducing pest numbers when their populations are diverse and abundant within an agroecosystem. Additionally, spiders possess characteristics and behaviors that might enhance their effectiveness as biological control agents. For example, spiders can easily survive when a particular pest insect's density is low. This allows spiders to establish themselves in habitats early before a pest population has had the opportunity to begin growing exponentially which may contribute to pest suppression by spider predation (Roince et al., 2013). In addition to directly feeding on pest insects, spiders have been shown to have non-consumptive effects on pest communities (Hlivko and Rypstra, 2003; Rendon et al.,

2016). In these and other examples, the presence of spiders in a system can disrupt foraging by phytophagous insects and in some cases, insects are killed by spiders without being fed upon (Reichert and Maupin, 1998). These aspects of spider biology and behavior lend to their usefulness in conservation biological control. Key examples of pest predation, non-consumptive effects, and pest population suppression by spiders within major crops have been revealed by numerous studies (Table 1.3).

Table 1.3: Summary of studies highlighting the direct predation and/or indirect disturbance of insect crop pests by spiders in major crops.

Crop	Insect Pest	Scientific Name	Pest Life Stage	Effect Type	Spider guild(s) responsible	Effect on Pest/Crop	Source
Alfalfa	Leafhoppers, aphids	Hemiptera: Cicadellidae, Aphididae	Adults	Consumptive	Ground Hunters	Direct predation	Yeargan (1975)
Orchards	Aphids	Hemiptera: Aphididae	Adults	Consumptive	Foliage Runners, Ambushers, Orb web	Early season aphid abundance negatively related to increased spider abundance	Roince et al. (2013)
Corn	True armyworm	*Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae)	Larvae	Consumptive	Ground Hunters	Damage to corn plants increased with predator removal	Clark et al. (1994)
	Southwestern corn borer	*Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae)	Eggs, larvae	Consumptive	Ambushers, Wandering sheet-web	Direct predation	Knutson & Gilstrap (1989)
	Beet armyworm	*Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)	Eggs	Consumptive	Foliage Runners, Wandering sheet-web	Direct predation	Pfannenstiel (2008)
	Corn earworm	*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)	Eggs	Consumptive	Foliage Runners, Wandering sheet-web	Direct predation	Pfannenstiel (2008)
Cotton	Cotton fleahopper	*Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae)	Adults	Consumptive, Non-consumptive	Foliage Runners, Ambushers	Direct predation	Breene et al. (1990)
	Beet armyworm	*Spodoptera exigua* (Lepidoptera: Noctuidae)	Eggs	Consumptive	Foliage Runners, Wandering sheet-web	Direct predation	Pfannenstiel (2008)
	Corn earworm	*Helicoverpa zea* (Lepidoptera: Noctuidae)	Eggs	Consumptive	Foliage Runners, Wandering sheet-web	Direct predation	Pfannenstiel (2008)

	Cotton bollworm	*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)	Larvae, adults	Consumptive, Non-consumptive	Ground Hunters	Direct predation; pest foraging disruption	Rendon et al. (2016)
Soybean	Velvetbean caterpillar	*Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae)	Adults	Consumptive	Foliage Runners, Ambushers, Orb web	Direct predation	Gregory et al. (1989)
	Japanese beetle	*Popillia japonica* (Newman) (Coleoptera: Scarabaeidae)	Adults	Non-consumptive	Space web, Orb web	Pest foraging disruption	Hlivko & Rypstra (2003)
	Mexican bean beetle	*Epilachna varivestis* (Mulsant) (Coleoptera: Coccinellidae)	Adults	Non-consumptive	Space web, Orb web	Pest foraging disruption	Hlivko & Rypstra (2003)
	Beet armyworm	*Spodoptera exigua* (Lepidoptera: Noctuidae)	Eggs	Consumptive	Foliage Runners, Wandering sheet-web	Direct predation	Pfannenstiel (2008)
	Corn earworm	*Helicoverpa zea* (Lepidoptera: Noctuidae)	Eggs	Consumptive	Foliage Runners, Wandering sheet-web	Direct predation	Pfannenstiel (2008)
Wheat	English grain aphid	*Sitobion avenae* (F.) (Hemiptera: Aphididae)	Adults	Consumptive	Ground Hunters, Ambushers	Early season aphid abundance negatively related to increased spider abundance	Birkhofer et al. (2008)
	Aphids	Hemiptera: Aphididae	Adults	Consumptive	Space-web, Wandering sheet-web	Direct predation	Chapman et al. (2013)
	Russian Wheat aphid	*Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae)	Adults	Consumptive	Orb-web, Ground Hunters	Direct predation	Kerzicnik et al. (2012)

When assessing spider potential for pest suppression, there are additional important factors that must be considered. For example, spiders are cannibalistic and will feed on other spiders as well as on other beneficial arthropods, a behavior shown to be beneficial to spiders (Michalko et al. 2020). Additionally, the efficacy of spiders to reduce pest populations is dependent on crop type and increased spider diversity has a positive yet small impact on pest suppression (Michalko et al. 2019). Evidence also suggests that spider guild and target pest type play an important role in pest suppression (Michalko et al. 2019). Finally, recent studies highlighting the non-consumptive effects of spiders in agroecosystems show that the impact of spiders on pest populations may

have been underestimated in the past when only direct predation was considered (Michalko et al. 2019).

Annual agroecosystem spider communities

Within the United States, annual crops, including corn, cotton, soybean and wheat, make up the majority of field crops produced, with corn being the largest overall (USDA, 2020). In 2020, 90.9 million acres of land in the U.S. were planted to corn, while cotton, soybean and wheat were planted to 12.1, 83.1 and 44.3 million acres, respectively (USDA, 2020). As annual crops, these agroecosystems are subjected to annual disturbance through planting and harvest, as well as through agronomic management throughout the growing season. These factors can contribute to significant reductions in arthropod biodiversity and abundance (Attwood et al., 2008). Despite this, spiders are found in all major annual cropping systems and can therefore provide a variety of ecological benefits within these systems.

The guild structure of spider communities within annual crops is dependent on crop type, although similarities across crops can be seen (Figure 1.1). Some guilds, including the wandering sheet-web, space-web, and ground runner guilds are commonly found across all crop types while foliage runners are found more frequently in cotton. Additionally, ambusher hunters make up a larger portion of the spider guild community in cotton, soybean and wheat than in corn. These differences and similarities illustrate the habitat preferences of spider guilds in agroecosystems.

Spider predation of or indirect impact on pests within annual crops has been fairly well documented. In corn, direct predation of the larvae of lepidopteran pests including true armyworm (Clark et al., 1994) and southwestern corn borer (Knutson and Gilstrap,

1989) have been observed. Additionally, the removal of spiders led to an increase in feeding damage to corn plants (Clark et al., 1994). This indicates some level of lepidopteran pest suppression by the spider community. Spiders have also been observed feeding on the eggs of lepidopteran pest species in corn, cotton and soybean (Pfannenstiel, 2008). While direct predation is the primary way in which spiders affect insect populations, indirect non-consumptive effects can play a significant role. For example, the presence of spiders caused a disruption in feeding by the cotton bollworm in cotton (Rendon et al., 2016) as well as the Mexican bean beetle and Japanese beetle in soybean (Hlivko and Rypstra, 2003). Although Thomisidae (crab spiders) and Salticidae (jumping spiders) are generally less abundant (or possibly less frequently sampled) in corn, predation of western corn rootworm beetles (*Diabrotica virgifera virgifera*, LeConte) by these spiders has been observed in the field (Figure 1.4). These examples of pest predation and disturbance by spiders provides evidence of their importance within annual crop fields.

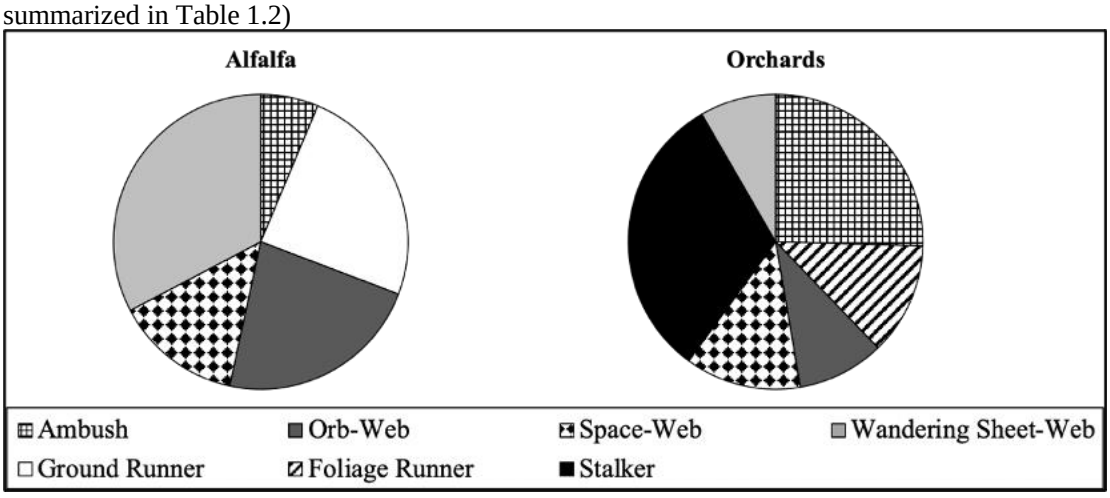
Perennial agroecosystem spider communities

While perennial agroecosystems are subjected to potentially disruptive management practices, including pesticide applications, their semi-permanence often provides more stability than annual systems. Additionally, perennial crops often support increased biodiversity and support ecological processes including methane consumption, pest suppression and pollination (Werling et al., 2014). The spider communities within perennial agroecosystems are often more diverse than those of annuals. Alfalfa and orchards make up a significant portion of land planted to commercial perennial crops, with 16 million acres planted to alfalfa and 5.6 million acres planted to orchards (USDA,

2020). For this reason, alfalfa and orchard systems can provide excellent examples of the spider community found within perennial agroecosystems.

Alfalfa is dominated by the wandering sheet-web, ground runner and orb-web guilds while orchard spider communities are primarily composed of the stalker and ambusher guilds (Figure 1.3). The vast difference in habitat structure between alfalfa fields and tree orchards accounts for the differences in guild composition.

Figure 1.3: The guild composition of spider communities in major perennial crops (Data from studies summarized in Table 1.2)



Research has shown that spiders can have a significant impact on pests within alfalfa fields and orchards. The presence of a diverse foliage-dwelling spider community in orchards can reduce early season abundance of aphids (Roince et al., 2013) while ground hunters have been found to prey upon leafhoppers and aphids within alfalfa (Yeargan, 1975). Non-consumptive effects have also been observed. In addition to direct predation, the presence of a foliage dwelling spider disrupted the feeding of a lepidopteran pest in apple (Mansour et al., 1981). These examples illustrate the importance of spiders in perennial systems; however, the incredible diversity of perennial crops require a systems-based analysis to determine the potential impact of the spider community.

Impact of agronomic practices on spider populations

Cropping systems throughout the world are subjected to disturbances from agronomic management activities including chemical, mechanical and cultural controls used within IPM programs. In addition to agronomic management practices, transgenic crops containing insecticidal *Bt* proteins are increasingly being utilized to manage insect pests. During the 2020 U.S. growing season, 88% of acres planted to cotton and 82% of acres planted to corn contained *Bt* traits (USDA, 2020). When considering the potential benefits of spider communities within agricultural systems, it is important to understand the ways in which crop management techniques impact these communities and to what extent.

Chemical controls & transgenic crops

Chemical controls are an important tool within the integrated pest management toolkit; however, care should be taken to ensure the most selective and least toxic, yet effective chemistries are used. Data collected over the past 30 years indicates that acaricides and insecticides are generally more harmful to spiders than herbicides and fungicides (Pekár, 2012). Additionally, certain chemical classes cause higher mortality rates in spiders than others: organophosphates (e.g., chlorpyrifos), pyrethroids (e.g., bifenthrin), and carbamates (e.g., carbaryl) (Pekár, 2012). These data highlight the overall impact of pesticides on spider communities as a whole; however, specific chemicals will affect spiders from different guilds in different ways. For example, a liquid insecticide sprayed on the top portion of the canopy may not come into contact with spiders at ground level, while web-builders located on the plant may not interact directly with soil-level treatments (Whitford et al., 1987; Haughton et al., 1999). Additionally, some pesticides can have negative effects on the physiology and behavior of spiders including

predatory behavior, web building, locomotion, and reproduction (Pekár, 2013). This shows that the impact of any given chemical on the spider community will depend on application method, active ingredient, and spider community guild structure. When selecting chemical treatments to use in the field, it is important to consider the possible effects the chemistries may have on spiders and other beneficial arthropods. Data describing the impact of the most commonly used insecticides and herbicides on spiders can be utilized as a guide for selecting chemical treatments that are less toxic (Table 1.4).

Table 1.4: The toxicological and behavioral impacts of common pesticides (herbicides and insecticides) on spiders. Risk was categorized as low (no detrimental effects), moderate (low mortality and/or detrimental impact on behavior) or high (moderate-significant mortality and/or detrimental effects from residues).

Pesticide Active Ingredient	Spider Guild(s) Affected	Effect(s)	Risk	Source
Acephate	Ground Hunter, Foliage Runner, Orb-web	Higher mortality in Orb-web guild	Low	Kumar and Velusamy, 1996
	Ground Hunter	No significant detrimental effects	Low	Thang et al. 1990
Acetochlor	Ground Hunter	Fresh, wet residues significantly reduced predatory activity	Moderate	Korenko et al. 2016
	Ground Hunter	One species more susceptible (20% mortality at field rate) than another species (10% mortality at field rate)	Low	Tahir et al. 2011
	Stalker	Low mortality (13%); reduced locomotion at initial exposure	Low	Tahir et al. 2012
Atrazine	Ground Hunter	Altered activity (slower movement, shorter courtship bouts)	Moderate	Godfrey and Rypstra, 2019
Bifenthrin	Space-web	Significantly toxic (~60% mortality); highest mortality 3 days post-application	High	Pekár et al. 2002
	Ground Hunter	Males more susceptible (30% mortality versus female 18% mortality at field rate); mortality from residues reduced with time	Moderate	Mukhtar et al. 2013
	Stalker, Ground Hunter, Foliage Runner	Population reduced by 86-92%, depending on rate	High	Kotha et al. 2014
	Foliage Runner	Courtship behavior disruption in males	Moderate	Hanna and Hanna, 2013
Carbaryl	Spider community	Most toxic to Araneae in this study	High	Kumar and Velusamy, 1996
	Foliage Runner	Courtship behavior disruption in males	High	Hanna and Hanna, 2013
	Ground Hunter, Ambusher, Orb-web, Stalker, Foliage Runner	Orb-web guild significantly reduced; ground spiders not impacted (timing, location of application)	Low-High	Whitford et al. 1987

	Spider community	Significant reduction in diversity and abundance, even 12 weeks after (residual)	High	Schulze et al. 2001
Chlorpyrifos	Foliage Runner	Highly toxic (100% mortality) even at lowest rate	High	Amalin et al. 2000
	Foliage Runner	Population suppression; 100% mortality in lab bioassay	High	Mansour, 1987
	Ground Hunter	Highly toxic (100% mortality) even 27 days post-application	High	Mustafa, 2011
	Ground Hunter	Negatively impacted growth; reduced abundance	High	Tahir, 2010
Glyphosate	Foliage Runner	Highly toxic (100% mortality) even at lowest rate	High	Amalin et al. 2000
	Ground Hunter	Significantly reduced predatory activity (wet residues)	Moderate	Korenko et al. 2016
	Web-builder, Ground Hunter	Highest rate reduced total number, web-builders specifically	Moderate	Haughton et al. 1999
	Ground Hunter	No significant detrimental effects on behavior	Low	Michalková and Pekár, 2009
	Orb-web	Significant detrimental effects on predatory behavior, web building, fecundity, fertility, and offspring development time	High	Benamú et al. 2010
	Ground Hunter	Interference with female pheromones leading to disruption in mate location ability in males	Moderate	Griesinger et al. 2011
Imidacloprid	Spider community	No significant detrimental effects	Low	Marquini et al. 2002
	Foliage Runner	Low (15%) to moderate (40%) mortality depending on formulation	Low-Moderate	Amalin et al. 2000
	Ground Hunter	Females produced fewer eggs; immatures had significantly higher development time; Low dosage increased predation	Moderate	Chen et al. 2012
Imidacloprid (seed treatment)	Spider community	No significant detrimental effects	Low	Albajes et al. 2003
λ-cyhalothrin	Stalker	51% mortality at field rate; significantly reduced locomotion and growth rate	High	Khan et al. 2018
	Wandering sheet-web	Inhibited spiderling emergence from egg sacs; delayed web building; high mortality	High	Dinter and Poehling, 1995
	Spider community	Significant reduction in Orb-web abundance; maximum effects observed 14-15 days post-application	High	Rodrigues et al. 2013

Currently within the United States, several types of genetically engineered (GE) crops are grown, including herbicide-tolerant soybeans, cotton, corn, sugar beets, canola and alfalfa; insect-resistant cotton and corn; virus-resistant papaya and summer squash; and with improved traits for transport and storage in apples and potatoes (USDA, 2020; FDA, 2020). These biotech crops have changed the way growers utilize chemical

controls. Herbicide-tolerant varieties have led to an increased use of herbicides while insect-resistant crops containing *Bt* proteins have contributed to a reduction in the use of insecticides (Benbrook, 2012; Klümper & Qaim, 2014). Due to the importance of GE crops within an IPM program, the impact of transgenic traits on the spider community must be evaluated to determine whether GE crops are compatible with efforts to support agronomic spider populations. Research has shown that spiders of various guilds are exposed to *Bt* endotoxins through both prey and crop pollen consumption (Peterson et al., 2016). Numerous studies evaluating the effect of these endotoxins on both foliar and epigeal spiders in a variety of crops have been conducted (Peterson et al., 2011). No significant deleterious effects have been detected in spiders inhabiting transgenic corn (Řezáč et al., 2006; Ludy & Lang, 2006); transgenic potato (Nedvěd et al., 2006); and transgenic cotton (Liu et al., 2006). Even more promising is the evidence that the adoption of *Bt* crops can lead to an increase in spider abundance and potential biocontrol services when reduced insecticide use is also implemented (Lu et al., 2012). The data from these studies indicates that *Bt* proteins expressed in transgenic crops are not harmful to spiders and can therefore be safely utilized alongside a conservation biocontrol program.

Other Agronomic Practices

In addition to the potential toxicological effects of chemical inputs, mechanical and cultural inputs as well as harvesting techniques can have a significant impact on the spider community. The primary mechanical control utilized to manage weeds is tilling, while cultural controls include habitat management, intercropping and cover crops. Methods used for harvest are also important to consider. Each of these crop management

methods have various effects on spider assemblages that must be considered when developing a conservation biocontrol program.

Tillage

Historically, tillage was a common practice to facilitate easy planting and for the control of weeds (Triplett and Dick, 2008). However, due to the potential negative impacts of tillage, including reductions in soil quality and crop yield (Nunes et al., 2018), no-till and reduced-till programs are being adopted at an increasing rate (Derpsch et al., 2010). Roughly 65% of land area within the U.S. planted to corn and 64% planted to soybean were under no or low-till management as of 2018 (USDA, 2020). The impact of tillage on beneficial arthropod communities, including spiders, is fairly well-studied. Because conventional tillage disturbs primarily the soil-surface, ground dwelling spider guilds are generally most affected. In corn managed with conservation rather than conventional tillage, ground hunters and wandering sheet-web builders exhibited increased abundance and species diversity (Schier, 2006). In a study examining tillage in wheat, however, the results were not consistent across three years. In the first year of the study, spider abundance decreased in conventional tillage fields versus reduced and no-till fields while in the following two years, the spider community was more abundant in conventionally tilled fields (Rice and Wilde, 1991). This highlights the complexity of agroecosystems and the importance of evaluating management programs as a whole. It may also indicate the different ways tillage impacts the spider community across crop types. Additionally, the ability of spiders to colonize new and disturbed habitats may account for the increased abundance in tilled fields.

Crop Diversity & Structural Complexity

Because spiders are generalist predators feeding on a variety of insect prey, it is important that a diverse number of spider species be present within an agroecosystem in order to benefit from the ecological services provided by different species (Marc & Canard, 1997). Spider species abundance and diversity within agricultural fields is primarily influenced by local factors, such as percent of ground cover, prey availability, and canopy cover (Marín et al., 2016). Various ground cover depths and types of canopy cover influence which spider guilds might be able to inhabit a given agroecosystem. For instance, web builders generally require vertical surfaces on which to attach their webs; therefore, crops that remain close to the ground may not support a diverse population of web-builders. The inclusion of non-crop habitats within or bordering crop fields can provide additional resources for spider communities and may enhance spider guild diversity. For example, wheat fields with a higher percentage of nearby non-crop habitat supported spider communities with greater species richness among the ambusher and space-web builder guilds (Pluess et al., 2010). Interestingly, species belonging to the wandering sheet-web builder, ground hunter and stalker guilds were not significantly affected by non-crop habitat landscapes in this study (Pluess et al., 2010). This may indicate the reliance of some guilds and not others on non-crop habitat to colonize crop fields. Research has also shown that even small patches of non-crop habitat can provide harborage for an increased abundance of spiders as well as unique species not commonly found in arable fields (Knapp and Řezáč, 2015). Not only do these habitats provide spiders with additional sources of prey and areas for harborage, but they may also provide an additional source of nutrition. Various species of spiders have been observed

feeding on pollen and nectar (Peterson et al., 2016; Chen et al., 2010; Taylor and Foster, 1996; Taylor and Pfannenstiel, 2008). Nectar has been shown to increase survival, growth and foraging activity in foliage dwelling spiders (Taylor and Bradley, 2009). The planting of non-crop habitats that include wildflowers and other sources of nectar may help to further support spider populations. In addition to non-crop habitats, the use of intercropping and cover crops can provide a variety of benefits including the enhancement of spider populations. The intercropping of alfalfa and cotton has been shown to facilitate a faster rate of increase in spider populations within cotton and a reduced rate of cotton aphid population growth (Lin et al., 2003). The increased habitat diversity of the alfalfa and cotton system provides refugia and additional prey sources for spiders. The implementation of cover crops can also benefit spider communities. In a study comparing a winter cover crop of grass versus bare soil in no-till corn fields, the predator communities were found to be structurally similar in both field types; however, greater predator abundance was found in the cover crop fields later in the growing season (Lundgren and Fergen, 2010).

Harvesting

Harvesting generally causes significant habitat disturbance and can seriously impact spider populations. For example, research has shown that cutting in alfalfa leads to an overall density decrease of web-building spiders (Birkhofer et al., 2007) and harvesting in wheat reduces overall spider abundance (Opatovsky and Lubin, 2012). However, there are harvesting methods that are less detrimental to spider communities. A study was conducted evaluating the impact of two harvesting techniques on the spider community. Border-harvesting, where patches of alfalfa are allowed to remain in the field

uncut, resulted in a much higher abundance of spiders versus fields that were completely harvested. The border-harvested fields also provided habitat within the uncut alfalfa so that after harvesting, the spider population was able to recover more quickly (Summers, 1976). In corn, the use of a sheller picker for harvest which allowed corn residue to remain, provided overwintering sites for spiders within the foliage runner, ground hunter and space-web builder guilds (Plagans and Whitcomb, 1986). It is important to note, however, that the corn residue also provided refugia to corn pests, particularly lepidopterans (Plagans and Whitcomb, 1986). This further illustrates the need for an IPM program which utilizes a variety of tactics that work together to simultaneously manage pest populations while also safeguarding beneficial arthropod communities.

Western Corn Rootworm

Biology & ecology

The western corn rootworm (*Diabrotica virgifera virgifera*) is a major coleopteran pest of corn in the family Chrysomelidae. This beetle originated in the Central American country of Guatemala where it has been a corn pest for thousands of years (Gray et al. 2009). *D. v. virgifera* was first discovered in the western Great Plains in 1867 after which it spread eastward across what is known today as the U.S. Corn Belt (Gray et al. 2009). The costs of management and crop losses incurred from this pest are estimated to be over $1 billion USD annually (Spencer et al. 2009). *D. v. virgifera* is a univoltine insect that overwinters as eggs within the soil (Branson et al. 1981). Larvae hatch from the eggs at the end of May or beginning of June and are drawn to corn roots by the CO_2 emitted from the root tips (Sandall et al. 2003) Larvae will develop through three instars before pupating within the soil, after which adults will begin to emerge in

July and August (Sandall et al. 2003). Adult beetles will climb upwards on the corn plant and begin feeding on leaves, silks, pollen and sometimes kernels (Branson et al. 1981). Female beetles tend to emerge after males, allowing them to mate soon after emergence (Sandall et al. 2003). Following roughly two weeks of feeding on corn plants the females return to the soil to lay eggs and will die soon afterwards (Sandall et al. 2003).

Management

Management of *D. v. virgifera* can be challenging since a significant portion of the insect's life cycle is spent belowground. For over a century, one of the most effective control techniques has been crop rotation which interrupts the *D. v. virgifera* lifecycle and successfully reduces the beetle's population density (Spencer et al. 2009). In the late 1980s, however, *D. v. virgifera* in some areas of the eastern Corn Belt were discovered causing damage to the roots of first-year rotated corn (Gray et al. 1998). Unlike the northern corn rootworm (*Diabrotica barberi*) which circumvents crop rotation in some regions by laying eggs capable of extended diapause, *D. v. virgifera* females will sometimes exhibit a reduction in fidelity to ovipositing exclusively on corn and will lay eggs in fields of other crops such as soybean (Gray et al. 1998). In areas of strict corn and soybean rotations, this allows the eggs to overwinter in the soybean field and hatch the following spring when the field is rotated back to corn. This shows that reliance on a single management method can lead to adaptations in the target pest that can impart resistance to the pest.

In addition to crop rotation, transgenic *Bt* corn is another effective, yet limited, *D. v. virgifera* management tool. This type of corn has been engineered to produce a toxin that has been derived from the soil bacterium *Bacillus thuringiensis* and is currently the

primary transgenic crop planted in the United States (Gassmann et al. 2011). In order to combat the development of resistance in target pests, the Environmental Protection Agency (EPA) has developed regulations which require the planting of non-*Bt* refuges along with the *Bt* crop as part of an Integrated Resistance Management program (US EPA, n.d.). These refuges allow pest insects that have yet to develop any resistance to survive and reproduce with more resistant individuals. This in turn helps to dilute the resistance trait within the population, potentially keeping the trait recessive and therefore delaying complete resistance (Gassmann et al. 2011). Unfortunately, as many as 50% of US growers are not willing to risk any loss in yield that planting refuges may cause and therefore do not follow the EPA guidelines for refuges (Gassmann et al. 2011). This lack of compliance will only serve to accelerate pest resistance thereby reducing the efficacy of *Bt*. In Nebraska, *D. v. virgifera* populations have already evolved resistance to *Bt* traits Cry3Bb1 and mCry3a in the field (Reinders et al. 2018). This is further evidence that a multi-technique approach is necessary in the management of *D. v. virgifera*.

The application of soil insecticides has been widely used as a control technique for larval *D. v. virgifera*. Before the development of *Bt* hybrids, crop rotation and the application of soil insecticides were the primary control methods used to limit *D. v. virgifera* feeding damage. Today, soil insecticides are still used; however, they are generally less effective than other control methods (Johnson et al. 2017) and their ability to protect roots is dependent on a variety of uncontrollable factors (Sutter et al., 1989). In a study to evaluate the effectiveness of *Bt* hybrids and soil insecticides against *D. v. virgifera* larvae, Johnson et al. found that *Bt* hybrids are superior to non-*Bt* crops planted with soil insecticides. In fact, this study revealed that soil insecticides, when used in

conjunction with *Bt* hybrids, can reduce root feeding damage but only in areas of very high pest pressure (Johnson et al., 2017). Additionally, the capability of soil insecticides to protect roots is dependent on rainfall/soil moisture and pest population density (Sutter et al., 1989) as well as wind direction and speed at the time of application (Bergman et al., 1991). These limitations illustrate the need for additional control methods against larval *D. v. virgifera*.

When measures to control *D. v. virgifera* larvae are unsuccessful, adult-targeting insecticides are commonly utilized for the management of adult beetles. From the 1970s through the early 1990s, carbaryl and methyl parathion were effective against adult rootworms; however, by the late 1990s resistance to these chemicals was evident in populations of Nebraska rootworms (Meinke et al, 1998). Today a variety of products are labeled for the control of *D. v. virgifera* adults, many of which contain the following active ingredients: permethrin, beta-cyfluthrin, bifenthrin, chlorpyrifos and cypermethrin (Wright et al. 2015). Chlorpyrifos is part of the organophosphate class of insecticides while the remaining five are pyrethroids. Unfortunately, rootworm populations exist in Nebraska that are resistant to both pyrethroid (Pereira et al. 2015; Souza et al. 2019) and organophosphate (Scharf et al. 2000) insecticides.

Biological control

The western corn rootworm is thought to have originated in Central America and has been present in the western Great Plains since the 1860s (Gray et al. 2009). The search for natural predators has resulted in several studies, some with promising results.

Finding an effective biological control agent that can effectively reduce *D. v. virgifera* populations below economic levels has proven challenging. In one study by

Kuhlmann et al. (2005), *D. v. virgifera* eggs, larvae, pupae and adults from Central Europe were removed from the field and evaluated in the laboratory for the presence of parasitoids, nematodes and pathogens. Only two strains of pathogenic fungi were found within less than 1% of the tested specimens (Kuhlmann et al. 2005). This is a strong indication that natural enemies endemic to Central Europe are not significantly attacking *D. v. virgifera*. This same study surveyed the native range of *D. v. virgifera* in Central and South America for potential natural enemies that could be used in augmentative biological control efforts. Adult parasitoids, including two tachinid flies and one braconid wasp, as well as mermithid nematodes were found to be the primary natural enemies of *Diabrotica* beetles within their native habitat (Kuhlmann, et al, 2005). In the United States, a study conducted in Minnesota evaluated predation of larval *D. v. virgifera* by entomopathogenic nematodes (Journey and Ostlie, 2000). They found that nematodes were able to control *D. v. virgifera* populations; however, significant challenges still exist for real-world application (Journey and Ostlie, 2000). Prischmann et al. (2011) found that subterranean predatory mites do feed on *D. v. virgifera* larvae but may not be effective control agents unless the mite population is diverse. In Nebraska, entomopathogenic nematodes from the genera *Heterorhabditis* and *Steinernema* were found in corn fields (Oliveira-Hofman, 2018) and both have species capable of infecting rootworms (Toepfer et al. 2009). Cursorial predators may also feed on rootworm larvae or emerging adults; however, an additional study in Nebraska found that carabid beetles are not significant predators of *D. v. virgifera* in the field (Oliveira-Hofman et al 2020). The findings of these studies show that biocontrol of *D. v. virgifera* is a complex and challenging issue.

Currently, there are very few studies evaluating the potential of spiders to be effective biological control agents of *D. v. virgifera*. This is not surprising since *D. v. virgifera* is beneath the soil for much of its lifecycle and generally less available to spiders as prey. However, adult beetles could potentially serve as prey for spiders of a variety of guilds. One study was conducted in Hungary by Tóth et al. (2002) to determine spider predation of *D. v. virgifera*. Spiders within the families Agelenidae and Theridiidae did prey on adult beetles, however, spider abundance was low within the sampled fields (Tóth et al., 2002). Although spider densities were low in this study, additional research should be conducted in various maize cropping systems to determine if spiders can have a beneficial impact on agroecosystems under different field conditions. Lundgren et al., (2009) evaluated the predation of *D. v. virgifera* eggs and larvae in the field. Although only 166 of 1,550 arthropod predators analyzed tested positive for *D. v. virgifera* DNA, spiders were among the predators exhibiting a rather high frequency of *D. v. virgifera* detection (Lundgren et al., 2009). These data are encouraging and support the possibility of spiders being effective biological control agents of this beetle.

Western Bean Cutworm

Ecology & Lifecycle

The western bean cutworm (*Striacosta albicosta*) is a native lepidopteran pest of corn and dry beans within the family Noctuidae (Smith et al. 2019). Historically, *S. albicosta* was first described in the 1880s in Arizona (Smith, 1887) and by the 1950s was found throughout the western Great Plains, including Nebraska (Hagan, 1962). Since 2000, *S. albicosta* has greatly expanded its range to include 22 new states and portions of

Mexico (Sánchez-Peña et al. 2016) and Canada, even as far north as Nova Scotia (Smith et al. 2019). This moth is univoltine and adult flight generally begins in June and ends in August or September with peak flight occurring in July (Smith et al. 2019). Oviposition occurs in late June through July and eggs are laid on the top surface of leaves on the top half of the corn plant (Smith et al. 2019). Throughout July neonate larvae hatch from egg masses and eventually move up the plant to feed on tassel tissue (Paula-Moraes et al. 2012). As the larvae mature, they move down the plant towards the ear where they sometimes feed on silks before entering the ear to feed on kernels (Smith et al. 2019). It is at this stage that chemical controls become nearly ineffective because the larvae are protected within the ears of the corn plant. *S. albicosta* larvae develop through six, rarely seven, instars before dropping off the plant to burrow into the soil and complete the next stage of the lifecycle in late summer or early fall (Montezano, 2019). *S. albicosta* overwinters in the soil as pre-pupae before pupating the following spring (Smith et al. 2019).

Management

The primary methods of control for *S. albicosta* include foliar insecticides, *Bt* corn and biological control (discussed in the next section) with each method having both benefits and drawbacks (Smith et al. 2019). Significant yield losses of 3.7 bushels per acre can occur with only a single *S. albicosta* larva per plant (Appel et al., 1993). Because *S. albicosta* larvae are seldom cannibalistic, several larvae have the potential to infest the same plant and even the same ear, which can lead to devastating yield losses. However, plant to plant larval movement is common and often leads to the infestation of neighboring plants (Pannuti et al. 2016).

The application of foliar insecticides to control *S. albicosta* larvae requires adult trapping and field scouting for egg masses in order to ensure a timely and effective application (Paula-Moraes 2013). While timing of these applications is an important factor due to larval movement into the ear, it is not the only variable that impacts treatment efficacy (Swoboda-Bhattarai et al. 2019). In Nebraska, a survey conducted in December 2016 through February 2017 collected responses from growers and agronomic professionals (i.e., crop consultants) regarding *S. albicosta* management (Archibald et al. 2018). This study found that the majority (81%) of insecticides used in Nebraska to manage *S. albicosta* from 2014 - 2016 were pyrethroids containing the active ingredients bifenthrin and/or zeta-cypermethrin (Archibald et al. 2018). While field-evolved resistance to pyrethroids has not been detected in *S. albicosta* at this time, care must be taken in order to mitigate resistance development and to ensure the future efficacy of these chemistries.

Currently, the only *Bt* trait effective against *S. albicosta* is the Vip3a protein as the development of Cry1F resistance led to the removal of *S. albicosta* control from registered Cry1F products in 2017 (Unglesbee 2017). The resistance of *S. albicosta* to Cry1F has been detected throughout the U.S. corn belt and Ontario (Smith et al. 2017) and survivability of larvae exposed to the toxin is evenly distributed throughout this insect's range (Coates et al. 2020). This reliance on a single trait for *S. albicosta* control requires very careful resistance management procedures and highlights the need for a multi-modal management program.

Biological control

As a native insect of North America, the western bean cutworm has a number of natural enemies that prey upon eggs, larvae and adults. An early study found that adult Coccinellidae, *Orius insidiosus* nymphs, Nabidae adults and lacewing larvae all fed on *S. albicosta* eggs (Blickenstaff 1979). In Nebraska, molecular gut-content analysis was conducted on field collected predators to determine whether predation on *S. albicosta* was occurring (Archibald 2017). The results indicated that Coccinellidae, *Orius insidiosus*, and specimens representing two spider families (Salticidae and Tetragnathidae) preyed upon *S. albicosta* in the field (Archibald 2017). Additionally, parasitoid wasps from the genus *Trichogramma* have been known to manage *S. albicosta* populations (Smith et al. 2019). In addition to invertebrate predators, *S. albicosta* is also susceptible to predation by vertebrates and birds as well as infection from pathogens, including microsporidia in the genus *Nosema* (Krupke et al., 2009; Po-lun Su 1976).

Predation of *S. albicosta* by spiders has not been studied; however, there are a handful of studies evaluating the predation of other noctuid crop pests by spiders. A four-year study by Pfannenstiel (2008) evaluated the spider predators of the eggs of two noctuid moths within Texas agroecosystems. Eggs of *Helicoverpa zea* and *Spodoptera exigua* were placed in cotton, corn and soybean fields and subsequently observed for predation (Pfannenstiel, 2008). Over the four year period, a total of 1565 predation events were observed, 23.4% of which were committed by spiders (Pfannenstiel, 2008). Of all spiders observed preying on eggs, 86.1% belonged to the families of Anyphaenidae, Miturgidae and Linyphiidae (Pfannenstiel, 2008). Predation of eggs by spiders occurred more often in cotton fields (26.6%), while soybeans (15.4%) and corn (6.3%) exhibited

less frequent egg predation by spiders (Pfannenstiel, 2008). It is also interesting to note that both immature and adult spiders were observed consuming eggs and that the vast majority of spider predation events were nocturnal (Pfannenstiel, 2008). This study shows that spiders can be important predators of the eggs of noctuid pests. A second study, conducted by Clark et al., (1994), sought to determine the consumption of armyworm by ground-dwelling generalist predators. Lab feeding trials were conducted to determine the willingness of field collected predators to feed on armyworm larvae while a predator removal study sought to evaluate how the presence of predators impacts armyworm herbivory (Clark et al., 1994). During the lab feeding trials, lycosid spiders did consume all armyworm larvae offered which indicates the spiders' ability to utilize armyworm larvae as prey (Clark et al., 1994). For the predator removal study, pitfall traps were placed in removal areas of a cornfield in order to reduce the density of predators within the removal sites (Clark et al., 1994). Corn plants within these removal areas were then visually examined for armyworm feeding damage and they found that these plants exhibited over twice as much damage as the plants outside of the removal area (Clark et al., 1994). Since lycosid spiders represented 13% of the predators removed, it's possible that their presence impacts armyworm herbivory (Clark et al., 1994). This demonstrates that spiders can have a non-consumptive effect on lepidopteran pests, thus reducing herbivory without direct predation.

Molecular Gut-Content Analysis

The study of trophic interactions can provide valuable data that can be used to better understand ecosystems and the ways in which human activity, including agriculture, impact those systems (van der Putten et al. 2004). In agroecosystems, an

understanding of the relationships between invertebrate predators and prey can be particularly useful when developing biological control programs and can inform the recommendation of treatment methods. Invertebrate trophic relationships can be assessed in a variety of ways including direct observation, non-molecular prey remains analysis, cage experiments and molecular gut-content analysis (Birkhofer et al. 2017).

The study of arthropod trophic relationships in the field has been greatly enhanced by the development of molecular methods. One of the simplest and most cost-effective ways to determine predation is through DNA extraction and subsequent PCR and gel electrophoresis of extracted samples (King et al. 2008). Results from these analyses can reveal predator-prey interactions that may be impossible to detect through any other means (Symondson 2002).

While PCR-based gut-content analysis is very effective and one of the most commonly used techniques in these types of analyses, there are some limitations that must be addressed. Due to the sensitivity of PCR, contamination of specimens can lead to false positives, therefore overestimating the efficacy of a predator to reduce pest populations (King et al. 2008). A decontamination step before DNA extraction that includes rinsing the sample in 2.5% (or higher) bleach is effective at removing contaminants (Greenstone et al. 2012) and does not impact prey DNA detectability (Greenstone et al., 2011). The inclusion of positive and negative controls is also critical in detecting contamination and avoiding inaccurate PCR results (King et al. 2008). This type of analysis does not distinguish between predation and scavenging or secondary predation, so a positive result does not necessarily mean direct predation has occurred (Birkhofer et al. 2017). Results from PCR-based gut-content analysis cannot be

interpreted without the detectability half-life of the target prey DNA within the target predator. This is generally accomplished through laboratory feeding trials in which predators are fed target prey organisms and then killed at selected time intervals after feeding (King et al. 2008). Gut-content analysis is then performed on the predator samples and the time point at which 50% of the samples tested positive for target DNA is determined (McMillan et al. 2007). This process reveals the size of the DNA detection window for the predator and prey in question.

Numerous studies have been conducted to determine the predation of crop pests by spiders in the field. While spider predation of agronomic insect pests was highlighted earlier in this chapter, the variability of pest DNA half-life in spiders is an important factor and will be discussed here. The length of time that prey DNA is detectible in the guts of spiders is largely dependent on spider species and prey type. For example, the lycosid species (*Pardosa sternalis*) fed Russian wheat aphid exhibited a detectability half-life of 2.0 hours while *Tetragnatha laboriosa* (Tetragnathidae) fed the same prey had a half-life of 4.2 hours (Kerzicnik et al. 2012). In another example, linyphiid and tetragnathid spiders fed Collembola had half-lives of 32 hours and 9.5 hours, respectively (Chapman et al. 2013). Even spiders within the same genus can exhibit highly variable prey detectability when fed different prey. Lycosidae in the genus *Pardosa* that fed on aphids had a half-life of 3.7 hours (Kuusk et al. 2008) while *Pardosa* that were fed fruit flies had a half-life of 78 hours (Monzo et al. 2010). Some spiders have a significantly longer detectability half-life as is the case with *Tibellus oblongus* (Philodromidae) feeding on the leafhopper *Psammotettix alienus*, which had a half-life of 5 days (Fülöp et al. 2019). These studies illustrate the high variability of prey DNA detection windows in

spiders and further illustrate the need for feeding trials and the determination of detectability half-lives in gut-content analysis studies.

Rationale & Objectives

Currently, there are very few published studies evaluating the composition of spider communities in Nebraska, particularly in agroecosystems. Of the five published studies with Nebraska spider community data, only one sampled from agroecosystems (Worley, 1928). Additionally, the most recent comprehensive study of Nebraska spiders was conducted over 40 years ago (Rapp, 1980). Data from the current project will serve to fill in knowledge gaps regarding spider communities in Nebraska corn fields while simultaneously contributing to the catalog of known Nebraska spiders overall.

It has been demonstrated that spiders are co-inhabiting Nebraska agroecosystems with western corn rootworm and western bean cutworm (Oliveira-Hofman et al. 2018); however, the published data regarding spider predation of *D. v. virgifera* are limited (Lundgren et al. 2009; Lundgren and Fergen 2011; Lundgren and Fergen 2014) and non-existent for *S. albicosta*, although studies do exist that demonstrate spider predation of other noctuids (Pfannenstiel 2008; Rendon and Taylor 2016). The results from the second half of this project will reveal any trophic interactions that exist between spiders and pests of corn which can inform future conservation biocontrol programs.

The primary aim of this research is to determine the community structure of spider populations within Nebraska agroecosystems and the impact of management techniques; and to describe the trophic relationships between spiders and two key pests of corn within these agroecosystems. Therefore, the specific objectives of this project are as follows:

1. Describe the diversity and abundance of spider communities in western Nebraska corn agroecosystems under conservation and conventional management.

2. Determine the strength of the trophic relationship between spiders and western corn rootworm and western bean cutworm.

The results from this project will provide insight into the spider community composition within Nebraska agroecosystems, the specific spider guilds that may interact with corn pests in the field and whether significant predation of *D. v. virgifera* and *S. albicosta* is occurring.

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CHAPTER 2: Spider communities and the impact of agronomic practices in western Nebraska agroecosystems

Introduction

Agroecosystems have unique characteristics that do not exist in natural, unmanaged systems which can impact arthropod communities. When comparing the community structure of spiders, there is often a trend of higher diversity in natural systems versus managed systems while abundance is often not significantly different between the two ecosystem types (Roughley et al., 2006; Muma & Muma, 1949; Kerzicnik et al., 2013). This reduction in diversity can be attributed to two unique characteristics of cropping systems: habitat homogeneity and management practices. Agroecosystems often consist of monocultures that do not provide the habitat heterogeneity necessary to support a biodiverse fauna (Benton et al., 2003) and the various management practices and inputs applied to agroecosystems can be detrimental to potentially beneficial predatory arthropods (Thorbek & Bilde, 2004). Despite this, a number of studies have shown that the majority of agroecosystems, including corn, can support robust spider communities and therefore have the potential to benefit from the ecological services spiders provide (Nyffeler & Sunderland, 2003).

Unlike other beneficial arthropods, such as parasitoids and specialist predators, there is currently little evidence that a single spider species is capable of reducing a pest population (Riechert & Lawrence, 1997). However, spiders can be successful in reducing pest numbers when their populations are diverse and abundant within an agroecosystem. When attempting to describe the community structure of spider assemblages in agroecosystems it is necessary to consider crop types individually and to determine the

foraging strategies and habitat use of spiders to understand their potential impact on various insect crop pests. This can be done by grouping spiders that utilize the same resources within a habitat into ecological guilds (Jaksić, 1981). According to Uetz et al. (1999), spiders inhabiting agroecosystems can be grouped into one of eight guilds based on five ecological characteristics: foraging behavior, web type, use of microhabitat, level of mobility, and diel activity. Furthermore, the eight guilds can be grouped broadly into two main categories: hunting spiders and web-builders (Uetz et al., 1999). Each guild exhibits not only unique hunting strategies, but also tend to prefer different locations within a crop habitat. Spider communities vary in their guild structure between crop types, which is important when considering potential interactions between spiders and insect crop pests. The importance of the spider guild structure is evidenced by the vertical spatial distribution and wide variety of hunting strategies exhibited by spiders, which allows them to prey on insects of various types, sizes, and developmental stages.

Cropping systems are subjected to disturbances from agronomic management activities including chemical, mechanical and cultural controls used within IPM programs. When considering the potential benefits of spider communities within agricultural systems, it is important to understand the ways in which crop management techniques impact these communities and to what extent. Current research suggests that the application of insecticides (Pekár, 2012) and tillage (Schier 2006) can each have a significant impact on the spider community. Insecticides can have a variable effect on spiders since the following chemical classes cause higher mortality rates than others: organophosphates (e.g., chlorpyrifos), pyrethroids (e.g., bifenthrin), and carbamates (e.g., carbaryl) (Pekár, 2012). It is also important to consider specific spider families as they

can be affected differently by the same insecticide (Whitford et al., 1987; Haughton et al., 1999). The impact of tillage on beneficial arthropod communities, including spiders, is fairly well-studied. Because conventional tillage disturbs primarily the soil-surface, ground dwelling spider guilds are generally most affected. In corn managed with conservation rather than conventional tillage, ground hunters and wandering sheet-web builders exhibited increased abundance and species diversity (Schier 2006). In the first year of a study examining tillage in wheat, however, spider abundance decreased in conventional tillage fields versus reduced and no-till fields while in the following two years, the spider community was more abundant in conventionally tilled fields (Rice and Wilde 1991). These data highlight the complexity of agroecosystems and the variability of agronomic management impacts on spider communities.

Studies of arthropod communities in agroecosystems are valuable tools for understanding arthropod pest complexes as well as the potential for biological control services from beneficial predatory or parasitic arthropods. There are currently few published studies concerning spider community composition within Nebraska, with the most recent population survey published 40 years ago (Rapp 1980). Additionally, there is currently no known published data on spider communities in western Nebraska corn fields, specifically. For this part of the study, the composition of spider communities as well as their abundance and diversity were determined. Agronomic management data was also collected for each field site and the impacts of tillage and insecticide application to the spider community was analyzed.

Materials and Methods

Field sites

During the 2017 growing season, spider sampling and prey availability surveys were conducted from 30 May to 21 August at eight corn fields within western Nebraska. In 2018, spider sampling and prey availability surveys were conducted from 31 May to 30 August at four corn fields. Field sites were selected based on the expectation of having agronomic characteristics considered to be “conventional” or “conservation.” Conventional fields are generally characterized by the planting of continuous corn, increased pest pressure leading to an increase in pesticide use and the use of disruptive management methods such as conventional tillage. Conservation fields are generally characterized by the use of crop rotation, non-crop habitat borders, reduced pesticide use and reduced or no-till practices. Field management and history data was collected through the use of a survey developed in Microsoft Excel and sent to the growers via email. This survey collected data on crop rotation, insecticide use, herbicide use, fungicide use, fertilizer use, irrigation, field border habitat type, cultural controls (tillage and cover crops) field histories and planting data. Growers returned the completed surveys via email or traditional mail.

Spider sampling

Summary

Spiders and target prey were collected from each field site using pitfall trapping, hand collecting and sweep netting along set transects. In 2017, field work occurred from May 22 through August 22 and was completed over two days with four field sites visited each day. Night collections (hand collecting only) were conducted on June 14 -15 and

July 26-27 between 10:00 pm and 3:00 am Mountain Standard Time. In 2018, field work occurred from May 31 through August 30 and was completed in one day with all four field sites visited in the same day. Night collections were conducted on June 28 and August 2. For each collection date, the order in which field sites were visited was randomized to avoid sampling bias. Collected spiders were placed in microcentrifuge tubes or vials with 95% ethanol and transported back to the lab where they were stored at -20 °C.

Transect location

Within each irrigated field, the wheel track made by the second, third or fourth tower of the central pivot irrigation system was used as a transect to ensure samples were taken far enough from the field edge. In the irrigated fields, the transects were located 60-175 meters from the field edge and then followed 90 meters in length along the circular pivot track (Figure 2.1 A). Non-irrigated field transects were located approximately 60 meters from the field edge and extended 90 m towards the west (Figure 2.1 B).

Figure 2.1 A: Irrigated field with location of pitfall traps (yellow stars) and transect/pivot track (red x's); **B**: Dryland field with location of pitfall traps (yellow stars) and flagged transect (red x's). Orange flags represent field entry points and white flags represent the beginning of transects.

Pitfall trapping

Along each transect, four pitfall traps consisting of an outer cup (Mountain Mixing Cup, 32oz) and inner cup (Pactiv Delitainer, 32oz) were placed 20 m apart and 1.6 m offset from the transect line in an alternating pattern (Figure 2.1, yellow stars). Pitfall traps containing approximately 300 ml of propylene glycol-based marine antifreeze (Splash Premium RV & Marine Antifreeze) were placed in the field and the contents were collected one week after the traps were set. There were a total of five sampling periods in 2017, with start dates of: May 22-23, June 12-13, July 2-3, July 24-25, and August 14 (all eight field sites visited on this date). In 2018, there were also five sampling periods with the following start dates: May 31, June 21, July 12, August 2, and August 22. Trap contents were placed in Whirl-Pak (Nasco) bags and transported back to the lab where the contents were processed for spiders.

Hand collecting

Along each transect, four collection points located approximately 20 m apart, and 1.6 m offset from the transect were marked with flags. At each collection point, spiders were collected by hand for a timed total of two minutes: one minute spent searching near the ground and looking under any debris; one minute walking and disturbing larger pieces of debris and low vegetation. This collecting process is an adaption of the Coddington protocol (Coddington et al. 1991). Very small spiders on the ground and on corn plants were collected using an aspirator. Additionally, three consecutive corn plants 2 – 4 m offset from the transect line were randomly selected at each collection point and thoroughly examined for spiders.

Sweep netting

In 2017, a sweep sample was taken from each field during the early stages of vegetative corn growth. This was accomplished by walking while continuously sweeping along the transect for a total of 15 figure-eight patterned sweeps with a 111.76 cm diameter net. Sweep netting was abandoned later in the 2017 season and eliminated entirely in 2018 due to the height and density of the corn plants making this approach unfeasible.

Spider identification, diversity & abundance

Identification

All field collected adult spiders were identified to the family and genus level using "Spiders of North America – An Identification Manual" (2nd edition, Ubick et al., 2017) and to species level using published keys reviewing genera from Clubionidae (Dondale and Redner 1976c), Corinnidae (Reiskind 1969), Dictynidae (Chamberlin and Gertsch 1958), Gnaphosidae (Platnick and Shadab 1982; Platnick and Dondale 1992), Linyphiidae (Dupérré 2013; Prentice and Redak 2013; Crosby and Bishop 1928a; Dondale 1959; Miller 2007; Millidge 1983), Lycosidae (Dondale and Redner 1990), Oxyopidae (Dondale and Redner 1990), Philodromidae (Dondale and Redner 1978), Phrurolithidae (Dondale and Redner 1982), Salticidae (Griswold 1987), Tetragnathidae (Dondale and Redner 2003), Theridiidae (Levi 1957a), Thomisidae (Dondale and Redner 1978) and Trachelidae (Platnick and Shadab 1974a). Taxonomic changes and updates were verified using the World Spider Catalog (World Spider Catalog 2021). Immatures were identified to family level only. Identifications were confirmed with the assistance of spider taxonomic specialists Dr. Paula Cushing (The Denver Museum of Nature and

Science, Department of Arachnology), Dr. Michael Draney (University of Wisconsin – Green Bay), and Dr. Marc Milne (University of Indianapolis). Voucher specimens of each species and both sexes (if available) were preserved in ethanol and given to the University of Nebraska State Museum to be included in the arthropod collections.

Data Analysis

To determine the effect of sampling method, sampling period and field site on the overall abundance of spiders, a PROC GLIMMIX model was used with a negative binomial distribution. To determine whether sampling method had a significant effect on the spider guilds collected for each method, a contingency table was created to evaluate the guild composition of each sampling method (SAS Institute, Inc. 2013). Whether a relationship exists between collection method (variable) and guilds collected (outcome) was determined using Fisher's exact test (SAS Institute, Inc. 2013). These same analyses were run to determine if the composition of guilds collected had a relationship with field identity and collection month.

To determine diversity values for each field site, identified specimen data were analyzed using the Shannon diversity index ($H' = -\Sigma\, pi \ln pi$) and species evenness for each field site was also calculated using the Shannon evenness index ($E_H = H/H_{max} = H/\ln S$) (DeJong 1975). Additional tests of these data were conducted: the Bartlett test of homogeneity of variances to ensure no statistical difference in variances and the two-sample t-test to determine whether significant differences between conservation and conventional field types exist. These calculations were conducted in R (R Core Team 2020).

To determine the effect of agronomic practice on spider species diversity and evenness, analyses were conducted to determine whether tillage, crop rotation or insecticide use had an effect on spider diversity and spider evenness. For the tillage and crop rotation variables which had two choices each (strip-till or no-till and crop rotation or none), a non-parametric Wilcoxon Two-Sample test was used along with an Exact two-sided test to account for low sample sizes and look for overall differences (SAS Institute, Inc. 2013). The insecticide variable had three choices (liquid in-furrow at plant, seed treatment, or none) and each option's effect on spider diversity and evenness was therefore analyzed using a non-parametric ANOVA, the Kruskal Wallis test, as well as an Exact two-sided test due to low sample sizes and to determine whether overall differences exist.

Results

Spider Community Results

Spider Abundance

Over the course of both collection periods and across all field sites, a total of 1011 spiders were collected. Of the total, 300 (~30%) were immature specimens and therefore identified to family level only. The remaining specimens were comprised of 53 species in 34 genera and 15 families (Table 2.1). Pitfall trapping collected 31 unique species, five species were hand collected from the ground only and two species were collected exclusively by hand from the corn plants (Table 2.1).

Table 2.1 Spider species collected for this study. Species listed in bold are not currently listed in any published literature confirming their presence in Nebraska

Family	Genus	Species	Collection Method	Total # Collected
Agelenidae				
	Immature	--	HG, PF	5
Clubionidae				

	Clubiona	*abboti* (L. Koch)	HG, PF	4
	Clubiona	*kastoni* (Gertsch)	HG, PF	2
Corinnidae				
	Castianeira	*descripta* (Hentz)	PF	1
	Castianeira	*trilineata* (Kaston)	HG	1
Dictynidae				
	Argenna	*obesa* (Emerton)	PF	15
	Immature	--	PF	1
Gnaphosidae				
	Drassyllus	*depressus* (Chamberlin)	PF	1
	Drassyllus	*frigidus* (Banks)	PF	2
	Drassyllus	*nannellus* (Chamberlin & Gertsch)	PF	4
	Gnaphosa	***parvula*** (Banks)	PF	11
	Gnaphosa	*sericata* (L. Koch)	PF	2
	Gnaphosa	*sp.*	PF	1
	Zelotes	***pseustes*** (Chamberlin)	PF	2
	Zelotes	***puritanus*** (Chamberlin)	PF	1
	Immature	--	HC, HG, PF	12
Linyphiidae				
	Agyneta	***simplex*** (Emerton)	PF	1
	Agyneta	*unimaculata* (Banks)	HG, PF	132
	Eridantes	*erigonoides* (Emerton)	PF	14
	Erigone	***dentosa*** (O. Pickard-Cambridge)	HC, HG, PF	39
	Grammonota	*inornata* (Emerton)	PF	2
	Mermessus	*trilobatus* (Emerton)	PF	14
	Walckenaeria	***spiralis*** (Emerton)	PF	1
	Immature	--	HC, HG, PF	39
Lycosidae				
	Alopecosa	***aculeata*** (Clerck)	PF	2
	Arctosa	*rubicunda* (Keyserling)	PF	2
	Hogna	*frondicola* (Emerton)	HG, PF	2
	Hogna	*sp.*	HG	1
	Pardosa	***delicatula*** (Gertsch & Wallace)	PF	1
	Pardosa	***mulaiki*** (Gertsch)	HG	1
	Pardosa	*saxatilis* (Hentz)	HG	1
	Pardosa	*sternalis* (Thorell)	PF	15
	Pardosa	*sp.*	HG, PF	2
	Schizocosa	***aulonia*** (Dondale)	PF	3
	Schizocosa	*avida* (Walckenaer)	HG, PF	52
	Schizocosa	*bilineata* (Emerton)	PF	1
	Schizocosa	***communis*** (Emerton)	HG	1

	Schizocosa	*crassipalpata* (Roewer)	PF	5
	Schizocosa	***duplex*** (Chamberlin)	PF	1
	Schizocosa	***mimula*** (Gertsch)	PF	7
	Schizocosa	*ocreata* (Hentz)	HC, HG, PF	262
	Schizocosa	***retrorsa*** (Banks)	HG, PF	10
	Schizocosa	*sp.*	HG, PF	2
	Tigrosa	***annexa*** (Chamberlin & Ivie)	HG, PF	3
	Tigrosa	*grandis* (Banks)	HG, PF	2
	Tigrosa	*helluo* (Walckenaer)	HG, PF	5
	Tigrosa	*sp.*	HG	1
	Trochosa	*terricola* (Thorell)	HG, PF	13
	Varacosa	***shenandoa*** (Chamberlin & Ivie)	PF	1
	Immature	--	HC, HG, PF	208
Oxyopidae				
	Oxyopes	*salticus* (Hentz)	PF	1
Philodromidae				
	Thanatus	***formicinus*** (Clerck)	PF	3
	Tibellus	*oblongus* (Walckenaer)	HC, HG, PF	13
	Immature	--	HG, PF	6
Phrurolithidae				
	Phrurotimpus	*borealis* (Emerton)	PF	3
Salticidae				
	Chalcoscirtus	*sp.*	HC	1
	Habronattus	*sp.*	PF	3
	Talavera	*minuta* (Banks)	HG, PF	7
	Immature	--	HC, HG, PF	7
Tetragnathidae				
	Tetragnatha	*laboriosa* (Hentz)	HC	6
	Immature	--	HC, PF	3
Theridiidae				
	Asagena	*americana* (Emerton)	PF	2
	Theridion	*frondeum* (Hentz)	HC, HG, PF	7
Thomisidae				
	Mecaphesa	*celer* (Hentz)	Sweep Net	1
	Xysticus	***acquiescens*** (Emerton)	PF	1
	Xysticus	*ferox* (Hentz)	PF	14
	Xysticus	***gosiutus*** (Gertsch)	PF	1
	Immature	--	HC, HG, PF	19
Trachelidae				
	Trachelas	*tranquillis* (Hentz)	HG	1
	Trachelas	*sp.*	HG	1
TOTAL				1011

The impact of collection period, collection method and field site on overall spider abundance was determined. In 2017, the mean abundance of spiders collected was significantly affected by the collection period (DF = 9; F-value = 11.21; P = <.0001; Figure 2.2). Collection method and field site also had a significant impact on spider diversity in 2017 (Table 2.2). Since an interaction between method and field did not exist (P = 0.5947), differences within method and field were evaluated separately and significant relationships were found (Figure 2.3).

Figure 2.2 Mean number of spiders collected during each collection period in 2017. Means with the same letter are not significantly different. (DF = 9; F-value = 11.21; **P = <.0001**)

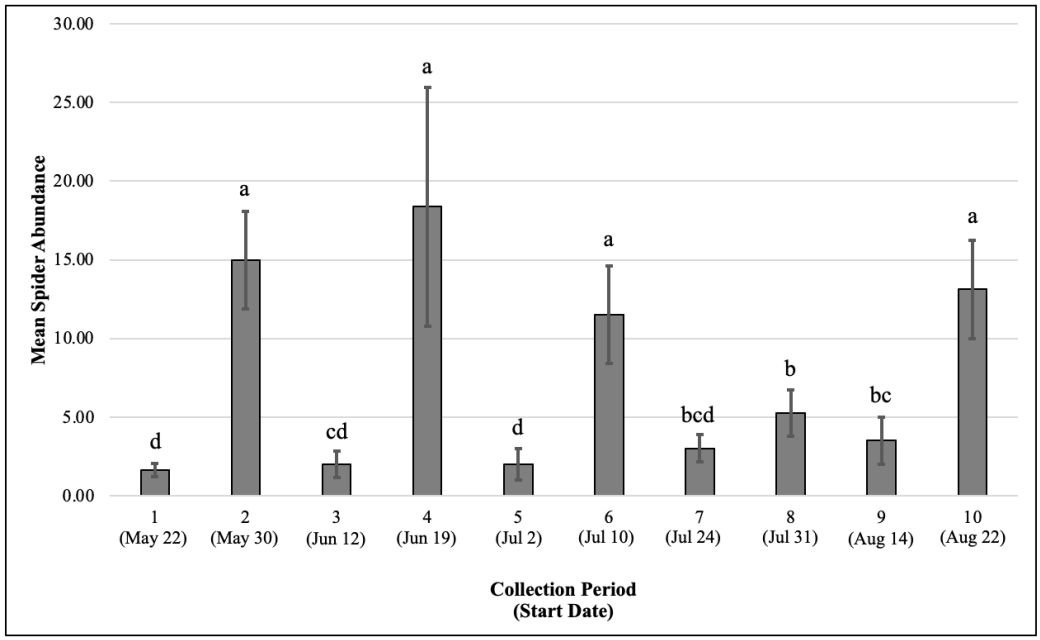
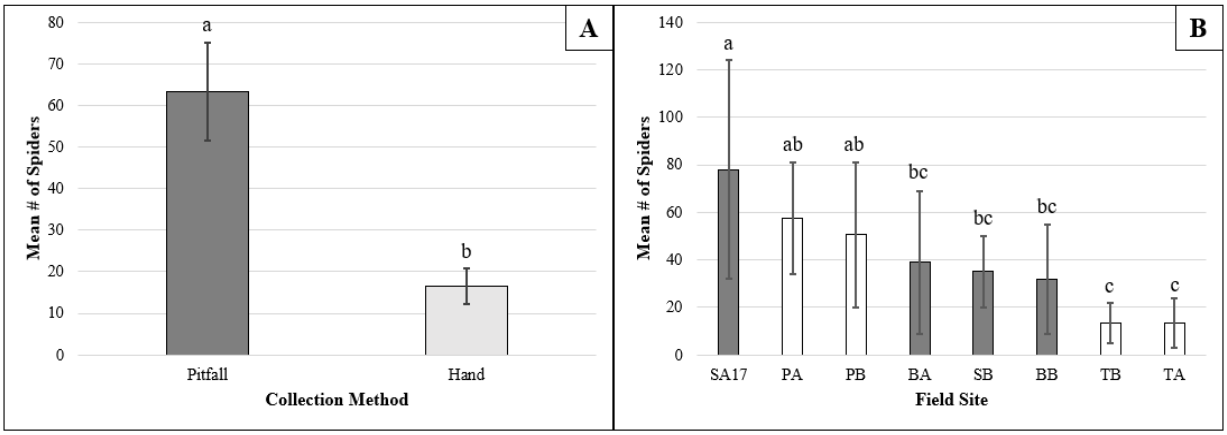


Table 2.2 Results of the PROC GLIMMIX analysis to determine effect of collection method and field on spider abundance in 2017

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Method	1	49	44.71	**<.0001**
Field	7	49	3.36	**0.0053**
Method*Field	7	49	0.80	0.5947

Figure 2.3 Mean number of spiders collected was significantly affected by collection method (A) and field site (B) in 2017. Means with the same letter are not significantly different. (Note on B: Conventional fields shaded in gray)



In 2018, collection period had a significant impact on mean spider abundance (DF = 9; F-value = 13.39; P = <.0001; Figure 2.4). In contrast to 2017, collection method and field site did not have a significant effect on mean spider abundance in 2018 (Table 2.3). Since an interaction between method and field did not exist (P = 0.5154), differences within method and field were evaluated separately; however, significant relationships were not found (Figure 2.5)

Figure 2.4 Mean number of spiders collected during each collection period in 2018. Means with the same letter are not significantly different. (DF = 9; F-value = 13.39; **P = <.0001**)

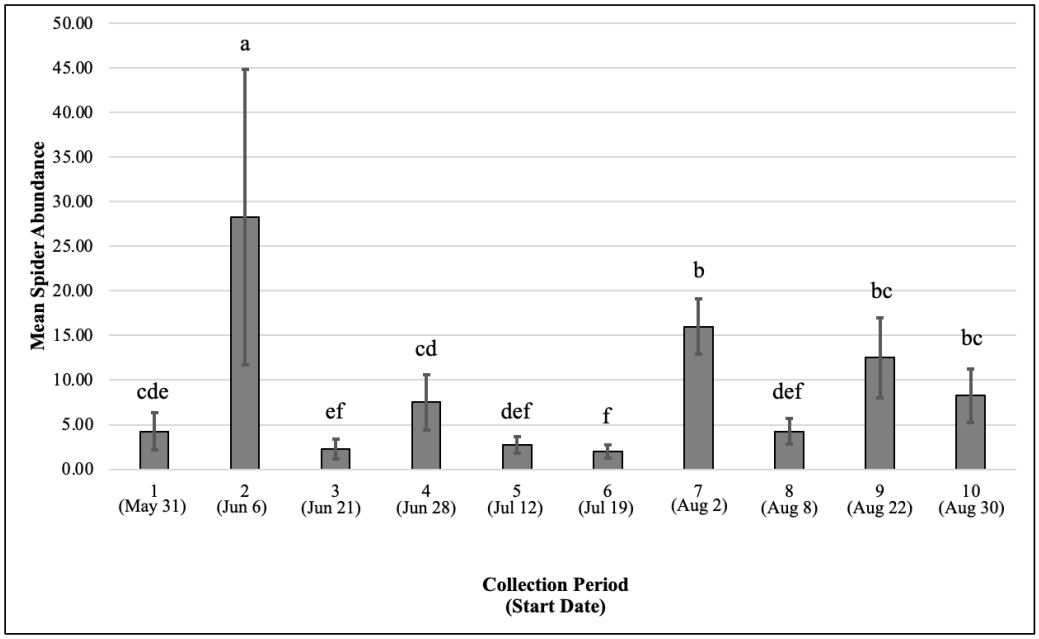
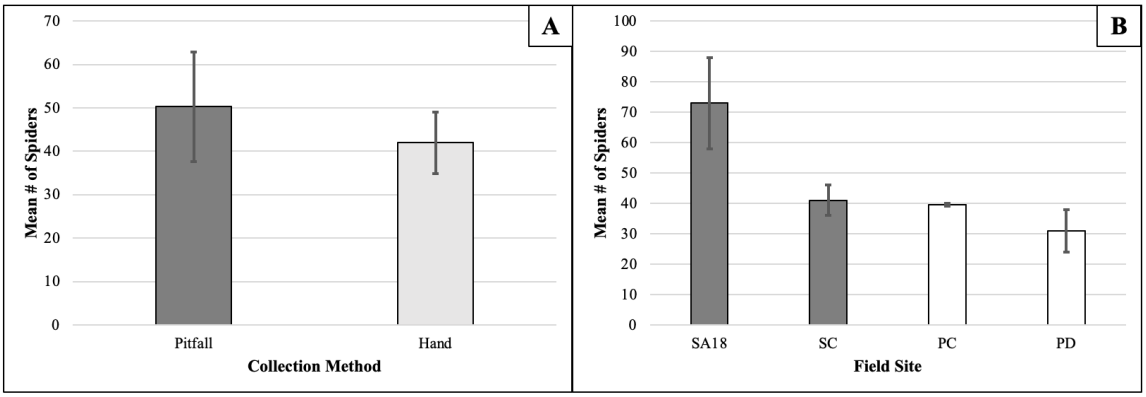


Table 2.3 Results of the PROC GLIMMIX analysis to determine effect of collection method and field on spider abundance in 2018

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Method	1	27	0.39	0.5362
Field	3	27	2.10	0.1235
Method*Field	3	27	0.78	0.5154

Figure 2.5 Mean number of spiders collected was not significantly affected by collection method (A) and field site (B) in 2018. (Note on B: Conventional fields shaded in gray)

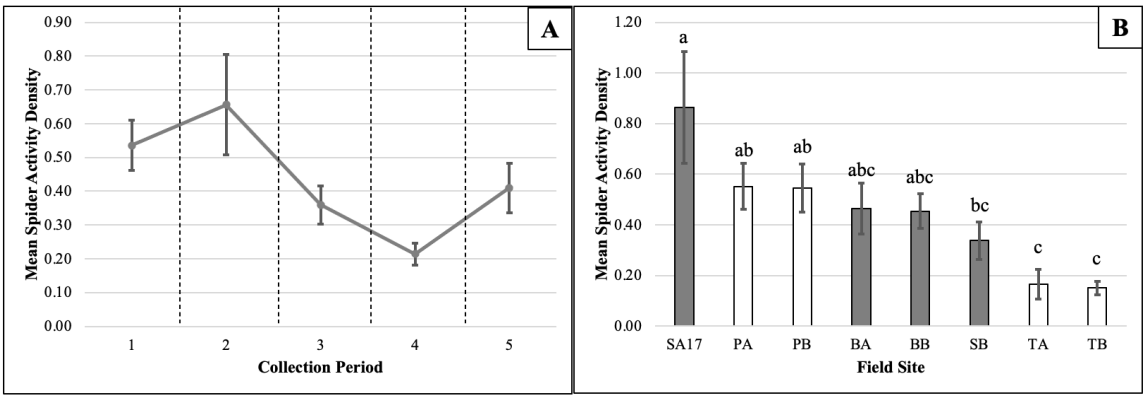


The impact of collection period and field site on pitfall-collected spider activity density was determined for 2017 and 2018. Since hand collected specimen data was pooled within each field site, further analysis was not conducted on hand collected spiders. In 2017, there was not an interaction effect between period and field (P = 0.9919) so the effects of field and period on spider activity density were evaluated separately (Table 2.4). While field site had a significant relationship with spider activity density, collection period did not (Figure 2.6).

Table 2.4 Results of the PROC GLIMMIX analysis to determine effect of collection period and field on spider activity density in 2017

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Period	4	144	1.87	0.1197
Field	7	144	2.17	**0.0404**

Figure 2.6 Mean activity density of spiders collected was not significantly affected by collection period (A) but was significantly affected by field site (B) in 2017. Means with the same letter are not significantly different. (Note on B: Conventional fields shaded in gray)

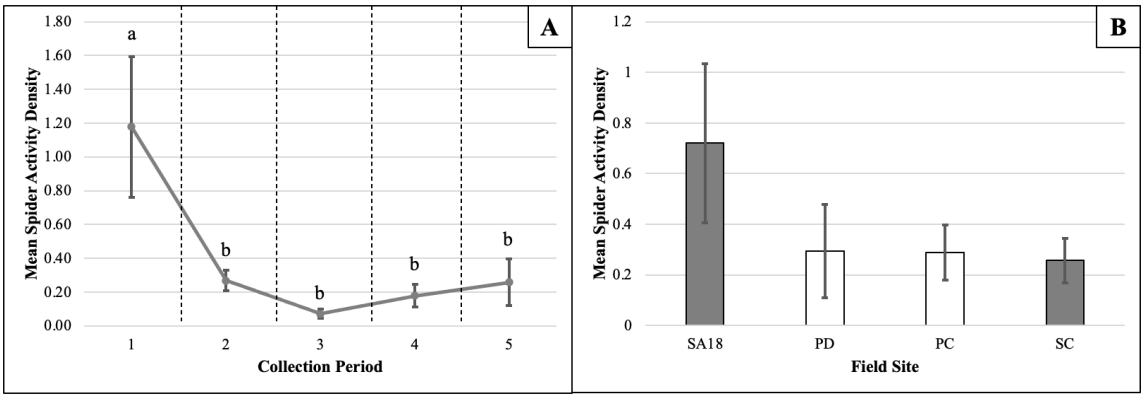


Similar to 2017, in 2018 there was not an interaction effect between period and field (P = 0.8971) so the effects of field and period on spider activity density were evaluated separately (Table 2.5). However, the impact of collection period and field site on spider activity density in 2018 was the opposite of the results found in 2017, with collection period having a significant effect and field site not (Figure 2.7).

Table 2.5 Results of the PROC GLIMMIX analysis to determine effect of collection period and field on spider activity density in 2018

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Period	4	72	5.25	**0.0009**
Field	3	72	1.62	0.1912

Figure 2.7 Mean activity density of spiders collected was significantly affected by collection period (A) but was not significantly affected by field site (B) in 2018. Means with the same letter are not significantly different. (Note on B: Conventional fields shaded in gray)



Community Composition

The relationship between spider guild composition and sampling method, field identity and month collected was determined. The guild composition of spiders collected by each method was affected overall by sampling method (DF = 40; F-value = 273.0303; P = <.0001; Figure 2.8). In addition, field identity (DF = 88; F-value = 422.3342; P = <.0001); Figure 2.9) and sampling month (DF = 24; F-value = 207.5238; P = <.0001); Figure 2.10) had significant overall effects on spider guilds collected.

Figure 2.8 The composition of spider guilds collected by each sampling method. The Fisher's exact test determined a significant overall relationship between collection method and guilds collected did exist (Fisher's p-value = <.0001).

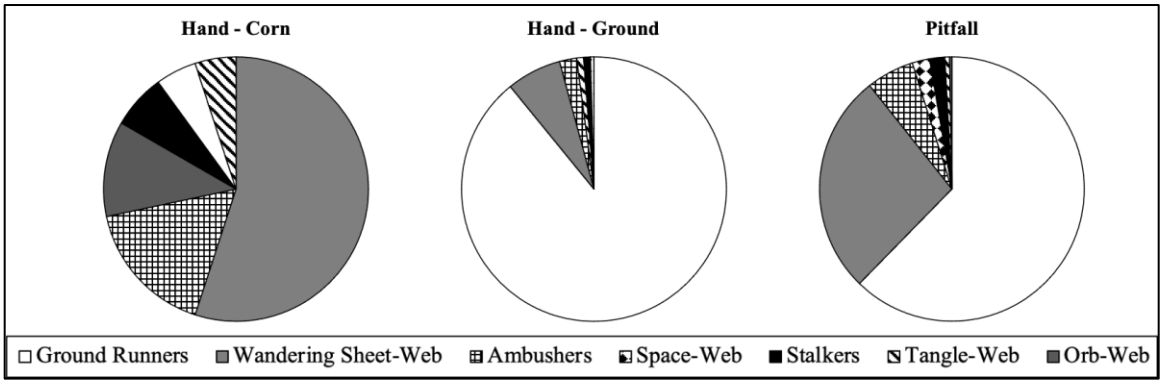


Figure 2.9. The composition of spider guilds collected from each field site. The Fisher's exact test determined a significant overall relationship between collection site and guilds collected did exist (Fisher's p-value = <.0001). Fields grouped by conservation-based and conventional management for visualization purposes.

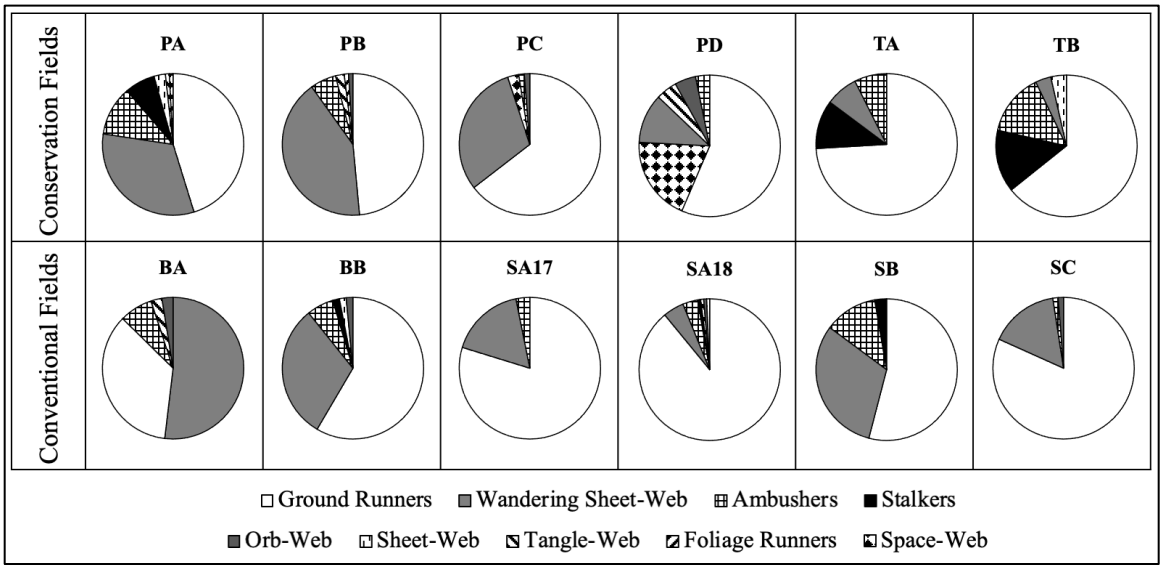
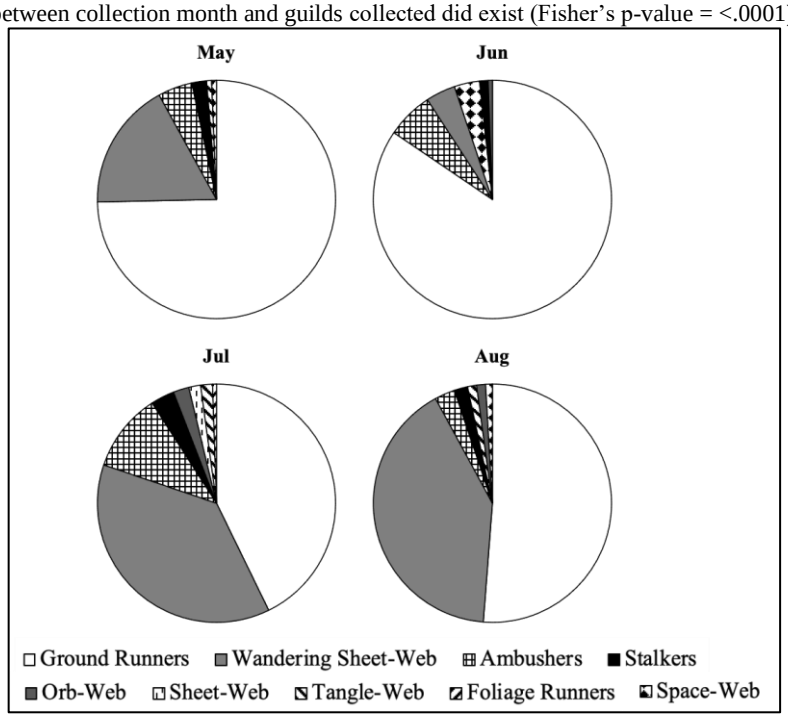


Figure 2.10 The composition of spider guilds collected in each month, both years combined. . The Fisher's exact test determined a significant overall relationship between collection month and guilds collected did exist (Fisher's p-value = <.0001).



Species Diversity & Evenness

For further community evaluation, spider diversity and evenness were calculated using the Shannon diversity (H) and evenness (E_H) indices. Species diversity indices ranged from 1.33 to 2.21 while species evenness values ranged from 0.49 to 0.93 with five of the six conservation fields exhibiting the highest diversity scores (Table 2.6). Species evenness was also highest among three of the conservation fields. One of the conventional fields sampled both years, SA17 and SA18, had some of the highest species richness values (15 and 16, respectively) but the lowest diversity (1.33 and 1.47, respectively) and evenness (0.49 and 0.53, respectively) values (Table 2.6).

Table 2.6 Spider community diversity, richness and evenness by field. Conservation fields shaded in gray.

Field	Shannon Diversity (H)	Species Richness	Shannon Evenness (E_H)
PA	2.21	18	0.76
PD	2.19	11	0.91
PC	2.18	14	0.82

PB	2.14	17	0.76
TB	2.04	9	0.93
SB	1.93	13	0.75
BA	1.79	15	0.66
SC	1.74	12	0.70
BB	1.67	9	0.76
TA	1.59	8	0.76
SA18	1.47	16	0.53
SA17	1.33	15	0.49

Field Characteristics and Impact

Field Site Results

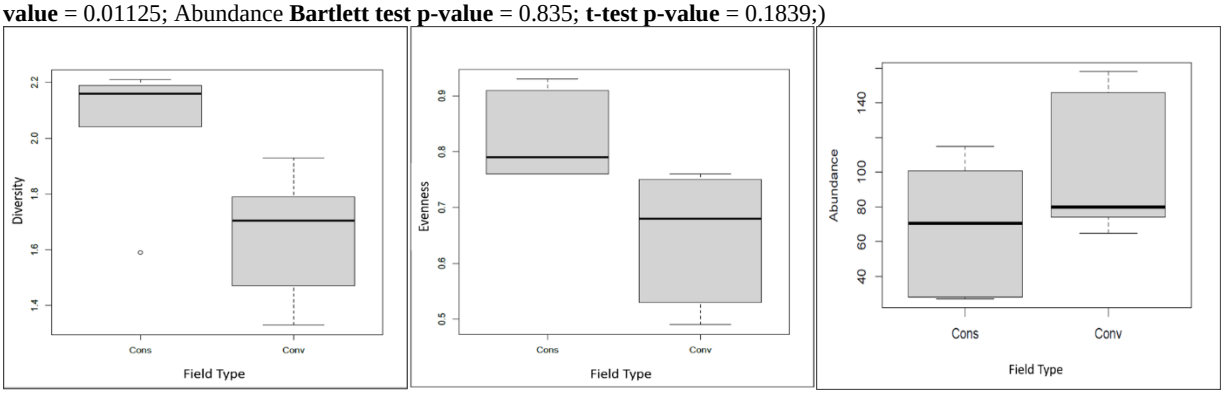
Questionnaires sent to growers were returned by email or mail and results were compiled and summarized (Appendix A, Table 2.7). The three agronomic practices that separate the field sites most evenly are tillage, crop rotation and insecticide use. Of the six conventionally managed sites, five were strip-tilled (BA, BB, SA17, SB, SC), one utilized crop rotation (BA) and four used insecticides (BA, BB, SA17, SB). Of the six conservation field sites, two were strip-tilled (PC, PD), four utilized crop rotation (PA, PB, TA, TB) and four used insecticides (PA, PB, PC, PD). Thus, a total of seven field sites were strip-tilled vs. five that were no-till, five utilized crop rotation and eight field sites used insecticides while the remaining four did not. Insecticide use can be further broken down into application method: four field sites used in-furrow insecticides at the time of planting, three field sites used treated seeds and one field site used both in-furrow insecticides and seed treatments.

Agronomic Impact

When comparing the spider diversity and evenness of the conservation fields to the conventional fields some significant differences can be seen (Figure 2.11). Both

spider species diversity and richness are significantly different between conservation fields and conventional fields (Figure 2.11). Spider abundance, however, was not significantly different between the two types of fields. For this reason, the impact of specific agronomic practices was analyzed for spider species diversity and evenness but not for spider abundance.

Figure 2.11 Spider species abundance, diversity and evenness of conservation versus conventional field sites. (Diversity **Bartlett test p-value** = 0.8691; **t-test p-value** = 0.01213; Evenness **Bartlett test p-value =** 0.4367; **t-test p-value** = 0.01125; Abundance **Bartlett test p-value** = 0.835; **t-test p-value** = 0.1839;)



The non-parametric Wilcoxon Two-Sample test along with an Exact two-sided test found no significant relationship between tillage and spider diversity (S = 34.00; p-value = 0.8763; No-till mean ± SEM: 1.890 ± 0.150; Strip-till: 1.832 ± 0.114) or tillage and spider species evenness (S = 36.50; p-value = 0.5619; No-till mean ± SEM: 0.749 ± 0.064; Strip-till: 0.729 ± 0.050) (Figure 2.12). Similarly, a significant relationship was not found between crop rotation and spider diversity (S = 38.00; p-value = 0.4318; Crop rotation mean ± SEM: 1.953 ± 0.116; No rotation: 1.787 ± 0.125) or crop rotation and spider evenness (S = 37.50; p-value = 0.4672; Crop rotation mean ± SEM: 0.775 ± 0.0437; No rotation: 0.710 ± 0.0575) (Figure 2.13).

Figure 2.12 The impact of tillage on spider species diversity (A) and spider species evenness (B) was not significant. (**Diversity**: S = 34.00; p-value = 0.8763; No-till mean ± SEM: 1.890 ± 0.150; Strip-till: 1.832 ± 0.114; **Evenness**: S = 36.50; p-value = 0.5619; No-till mean ± SEM: 0.749 ± 0.064; Strip-till: 0.729 ± 0.050)

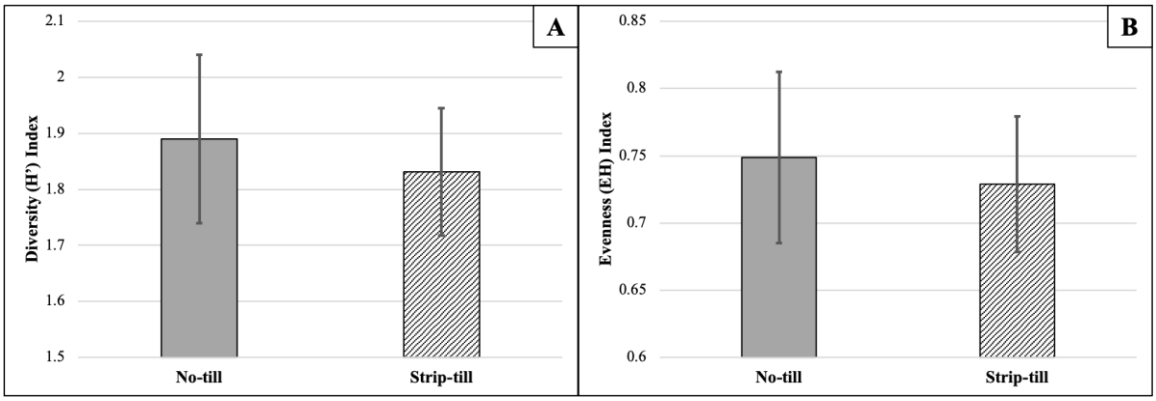
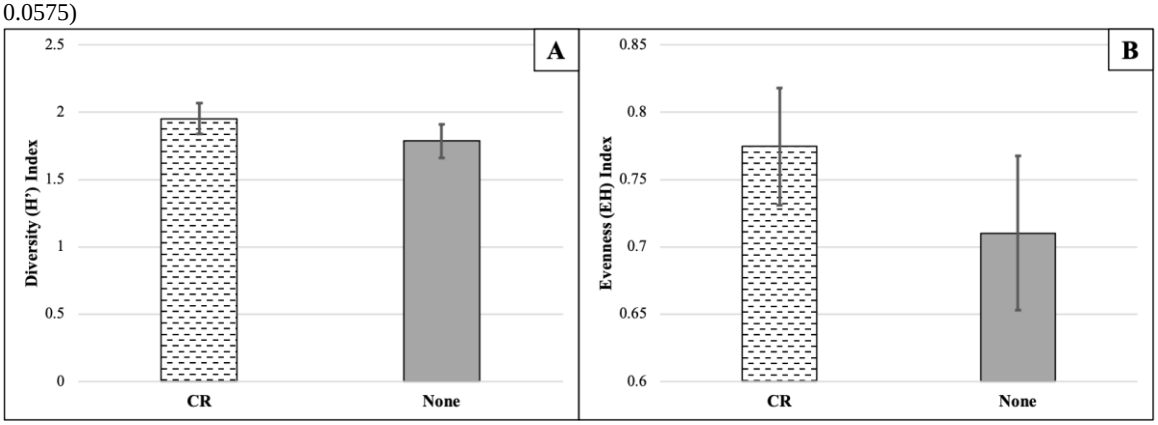
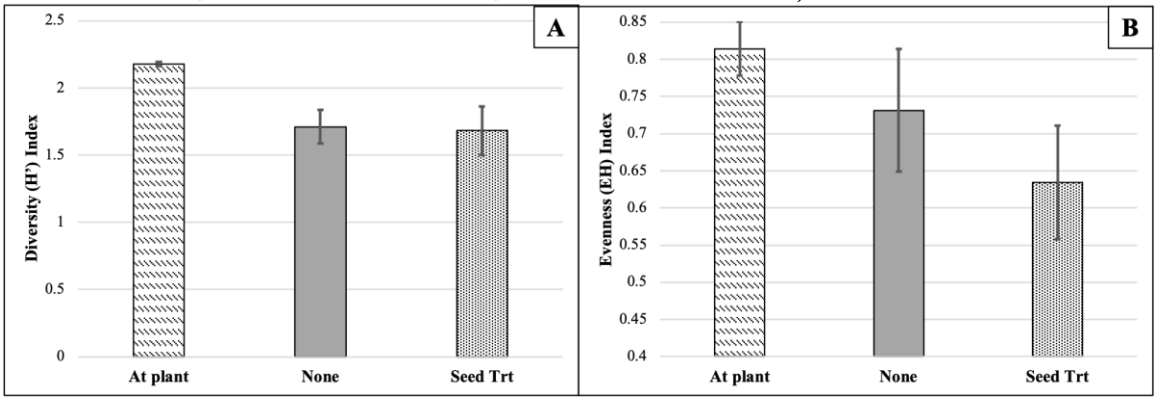


Figure 2.13 The impact of crop rotation on spider species diversity (A) and spider species evenness (B) was not significant. (**Diversity**: S = 38.00; p-value = 0.4318; Crop rotation mean ± SEM: 1.953 ± 0.116; No rotation: 1.787 ± 0.125; **Evenness**: S = 37.50; p-value = 0.4672; Crop rotation mean ± SEM: 0.775 ± 0.0437; No rotation: 0.710 ± 0.0575)



Insecticide use did show a significant relationship to spider diversity, but not to evenness (Figure 2.14). The Kruskal Wallis test revealed a significant overall relationship between insecticide use and spider species diversity (Chi-square = 7.00; DF = 2; **p-value = 0.0159**; At plant mean ± SEM: 2.178 ± 0.014; No insecticide: 1.711 ± 0.124; Seed treatment: 1.682 ± 0.181). A significant relationship between insecticide application and spider species evenness was not found (Chi-square = 4.38; DF = 2; p-value = 0.1216; At plant mean ± SEM: 0.814 ± 0.036; No insecticide: 0.731 ± 0.083; Seed treatment: 0.634 ± 0.076).

Figure 2.14 Insecticide use did show a significant relationship to spider diversity (A), but not to evenness (B). (**Diversity**: Chi-square = 7.00; DF = 2; **p-value = 0.0159**; At plant mean ± SEM: 2.178 ± 0.014; No insecticide: 1.711 ± 0.124; Seed treatment: 1.682 ± 0.181; **Evenness**: Chi-square = 4.38; DF = 2; p-value = 0.1216; At plant mean ± SEM: 0.814 ± 0.036; No insecticide: 0.731 ± 0.083; Seed treatment: 0.634 ± 0.076)



Discussion

During the course of this study, a total of 1,011 spider specimens composed of 54 species in 34 genera and 15 families were collected from the 12 field sites for both years combined. Of the total specimen count, 300 (30%) were immatures. Two families, Lycosidae and Linyphiidae, made up 60% (n = 605) and 24% (n = 244) of all specimens collected, respectively. Gnaphosidae (n = 36) and Thomisidae (n=36) were the third most common families collected, representing 4% each of all spiders collected. Many similar spider community surveys have been conducted with variable results. In corn fields in Germany, 1,811 foliage-dwelling spiders representing 50 species were collected via vacuum with Linyphiidae and Theridiidae being the most abundant families (Ludy and Lang 2006) while 5,181 ground spiders collected via pitfall traps were represented by 30 species and dominated by the families Linyphiidae and Lycosidae (Schier 2006). The collection of spiders using two sampling methods, pitfall traps and visual search with hand collecting, was conducted in a single corn field in India (Saranya et al. 2019). A total of 2,821 spiders consisting of 16 species in 10 genera and 6 families were collected, with Lycosidae making up 60% (n = 1671) of all specimens captured. A study conducted

using three sampling methods (refuge traps, dry pitfall traps and visual search and capture with an aspirator) collected a total of 1,108 spiders with 29 genera in 12 families, with Linyphiidae, Lycosidae and orb-web spiders the most common (Peterson et al. 2016). Each of these studies reported a higher overall count of total spiders collected, but a lower number of unique taxa compared to the current study. This is most likely a result of differences in sampling methods, sampling frequency and field site characteristics. The most commonly collected families in each study included Linyphiidae and/or Lycosidae regardless of collection method, which the current study also found to be the case. This illustrates a pattern of high lycosid and linyphiid abundance in corn fields. Linyphiidae and Lycosidae are abundant in agroecosystems most likely due to their dispersal and recolonization abilities (Oberg and Ekbom 2006). Additionally, lycosid abundance is often not significantly affected by habitat disturbance from agronomic management practices (Oberg and Ekbom 2006; Rendon et al. 2015).

Currently, studies evaluating the composition of spider communities in Nebraska are limited. The earliest survey of Nebraska spiders was published by Worley and Pickwell in 1927, with a short update published a year later (Worley 1928). These publications listed a total of 225 species in 109 genera and collections were made in primarily non-crop habitats including forest, prairie, sandhills, and high plains (Worley and Pickwell 1927) with some sampling in alfalfa (Worley 1928). A survey of tallgrass prairie spiders in eastern Nebraska collected a total of 5,311 spiders representing 111 species in 61 genera and 15 families (Muma and Muma 1949). The most recent comprehensive survey of spiders in Nebraska was conducted by Rapp in 1980 who reported a total of 344 species in 24 families (after compiling results with previously

published data). In 2014, eleven spider species were added to the Nebraska record (Nemec 2014) which, when combined with the Rapp 1980 data, results in a total of 355 species in 24 families known from published data to exist in Nebraska. If these numbers are accurate, the spider taxa found in western Nebraska corn fields are only a portion of the statewide taxa. Only 54 (15%) of the 355 known species and 15 (63%) of the 24 known families were collected from corn fields during the course of the current study. However, 19 species were collected during this study that are not listed in any currently published records of spider species in Nebraska.

Sampling method had a significant effect on the composition of spider guilds collected in the current study, which is similar to the results of previously published studies (Green 1999; Churchill and Arthur 1999; Costello and Daane 1997; Amalin et al. 2001; Hovemeyer and Stippicii 2000). Sampling the spider community from tree crop agroecosystems using a vacuum and pitfall traps resulted in significantly different spider assemblages (Green 1999). More spider taxa (81-97%) were collected by vacuum sampling from the tree foliage than by pitfall traps (29-57% of taxa) (Green 1999). Additionally, only 10% of species (n = 13) were collected using both methods. Similarly, only 7% (n = 4) of species in the current study were collected using all three methods. Churchill and Arthur (1999) compared the assemblage of spiders collected by pitfall traps, visual search and hand collecting, and sweep netting in a coastal habitat of Tasmania. A total of 8,625 spiders comprising 130 species in 33 families were collected, with pitfall traps collecting the most species (94%) while hand collecting resulted in the capture of 41% of species and sweep netting only collected 25% (Churchill and Arthur 1999). The results of these studies highlight the importance of utilizing more than a

single collection method when conducting arthropod community surveys. In addition to sampling method, sampling month also had a significant relationship with spider assemblages collected. This is in agreement with other published studies, including Churchill and Arthur (1999) who found significant temporal variation in the composition of spiders captured.

Spider species diversity and evenness was calculated using the Shannon Index (Spellerberg and Fedor 2003). In the current study, overall spider species diversity ranged from 1.33 (Field site SA17) to 2.21 (Field site PA) while evenness ranged from 0.49 (Field site SA17) to 0.93 (Field site TB). Species richness within each field site ranged from 8 species (Field site TA) to 18 species (Field site PA). Similar studies to determine spider species diversity in corn have found diversity indices as low as 0.54 – 1.01 (Silva et al 2014) and 0.45 – 1.13 (Pinkus-Rendón et al. 2006) to indices as high as 2.63 (Saranya et al. 2019). Spider species diversity and evenness were determined at three sites in eastern Colorado wheat agroecosystems, with species diversity ranging from 1.04 to 2.35 and species evenness from 0.47 to 0.87 across all three sites (Kerzicnik et al. 2013). These numbers are similar to those found in the current study. Non-crop habitats often support higher spider diversity. For example, in a mountain grassland habitat, spider diversity in hay meadows was found to be 2.80 to 3.10, although median diversity was higher in mowed (disturbed) meadows (Szmatona-Túri et al. 2019). In contrast, spider species evenness is not necessarily higher in non-crop habitats. A survey of ground-dwelling spiders in pine plantations and indigenous forests reported species evenness indices ranging from 0.54 to 0.83, a reduced evenness score compared to some agroecosystem field sites (Merwe et al. 1996).

While the impact of agronomic practices on spider communities has been extensively studied, the results reported here will help to create a more complete picture of how agronomic practices affect spiders. In the current study, there was no difference in spider diversity or evenness between no till and strip-tilled fields. This is most likely a result of strip tillage being a less destructive form of conservation tillage, wherein narrow strips of soil are tilled to prepare rows for seeding and generally less than 25% of the field area is disturbed (Morrison Jr. 2002). In addition, tillage can have variable impacts on arthropod communities. In a three-year study, Wenninger et al. (2020) found that species richness had an insignificant relationship with tillage while lycosid and linyphiid abundance was higher in strip-tilled versus conventionally tilled fields. This is a similar result to the current study in which strip-tilled field site SA had the highest mean spider abundance and activity density in both 2017 and 2018. In conservation tillage plots, the number of spider species was greater than in conventionally tilled plots (Schier 2006). The results of the current study may have been different had the comparison been between conventional tillage and no tillage or conventional tillage and conservation tillage.

There are currently few studies evaluating the impact of crop rotation on spider communities. The results of the current study found no significant relationship between crop rotation and spider diversity and evenness. In a study comparing the beneficial arthropod community in continuous corn, 2-year corn and soybean rotation, and 3-year rotation, diversity was significantly higher in continuous corn vs. the 2-year corn and soybean rotation (Dunbar et al. 2016). Meyer et al. (2019) conducted a 10-year crop rotation study to determine the impact on web spiders. They found that spider activity

density and species richness were significantly affected by the current year crop as well as preceding crops going back two years (Meyer et al. 2019). It is important to note, however, that Lycosidae and Linyphiidae did not show preference for any particular crop which may help to explain their abundance in many agroecosystems (Meyer et al. 2019).

In the current study, insecticide use was the only agronomic practice that showed a significant relationship with spider species diversity, but not species evenness. The effects of insecticides on spiders reported in the literature vary widely and are dependent on insecticide class, application method and spider guild (Chapter 1, Table 1.4). In the current study, none of the field sites were sprayed with foliar insecticides but rather utilized insecticide treated seeds and/or in-furrow applications. The majority of studies evaluating the impact of insecticides on spiders involve spray applications (Table 1.4) rather than systemic treatments, with a few exceptions. An area of grassland received an application of a soil insecticide (Chlorpyrifos) to determine its impact on soil-dwelling invertebrates (Fountain et al. 2007). While the diversity of Collembola was significantly reduced by the insecticide application, spider species diversity was not affected (Fountain et al. 2007). In a corn agroecosystem with neonicotinoid-treated seeds, the mean number and abundance of arthropod taxa (including spiders) collected by pitfall trap were higher in the plots with treated seeds; however, diversity was numerically but not statistically lower in plots with treated seeds (Disque et al. 2018). While this is similar to the results found in the current study where field site SA17/18 utilized treated seeds and also exhibited the highest mean spider abundance and activity density, other studies have found no significant impact on soil spider communities from the use of treated seeds (Albajes et al. 2003; Zhang et al. 2015; Dubey et al. 2019). Dubey et al. (2019) found that

foliar spider abundance was higher in fields planted with treated seeds and suggested that sublethal effects of the insecticides may have made prey easier to capture, thus increasing spider abundance. This may explain the increased spider abundance in fields that utilized systemic insecticides (in-furrow and seed treatments) in the current study.

The results of this study provide an increased understanding of the abundance and composition of spider communities in Nebraska agroecosystems and the impacts of agronomic practices on those communities. The data illustrating spider communities in Nebraska corn fields as well as species not previously described as existing in Nebraska are novel and contribute to current the knowledge base regarding arthropods in this state. This information is also critical for understanding the pest control potential of spiders in corn and the impact, or lack thereof, of agronomic practices on these communities in order to support their populations within agroecosystems.

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CHAPTER 3: Determination of the strength of the trophic relationship between spiders and key corn pests

Introduction

In agroecosystems, an understanding of the relationships between invertebrate predators and prey can be particularly useful when developing biological control programs and can inform the recommendation of treatment methods. Invertebrate trophic relationships can be assessed in a variety of ways including direct observation, non-molecular prey remains analysis, cage experiments and molecular gut-content analysis (Birkhofer et al. 2017). The study of arthropod trophic relationships in the field has been greatly enhanced by the development of molecular methods. One of the simplest and most cost-effective ways to determine predation is through DNA extraction and subsequent PCR and gel electrophoresis of extracted samples (King et al. 2008). Results from these analyses can reveal predator-prey interactions that may be impossible to detect through any other means (Symondson 2002).

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is a major coleopteran pest of corn in the family Chrysomelidae. *D. v. virgifera* was first discovered in the western Great Plains in 1867 after which it spread eastward across what is known today as the U.S. Corn Belt (Gray et al. 2009). The most significant damage from this pest is caused by larvae feeding on corn roots (Sandall et al. 2003) while adults can also interfere with pollination by feeding on silks (Branson et al. 1981). The costs of management and crop losses incurred from this pest are estimated to be over $1 billion USD annually (Spencer et al. 2009). Management of *D. v. virgifera* can be challenging since a significant portion of the insect's life cycle is spent belowground. The most

common methods of control are crop rotation, transgenic corn, soil insecticides and seed treatments for larval control and foliar insecticides for adult control. Unfortunately, some populations of *D. v. virgifera* have developed resistance to crop rotation (Gray et al. 1998) and populations have been detected with field-evolved resistance to *Bt* traits Cry3Bb1 and mCry3a in several states, including Nebraska (Reinders et al. 2018) as well as Cry34/35 in Iowa and North Dakota (Calles-Torrez et al. 2019; Gassmann et al. 2020). As for chemical controls, soil insecticides for larval control often have limited efficacy (Johnson et al. 2017) and the majority of foliar insecticides labeled for adult control are pyrethroids to which some Nebraska populations of rootworm have developed resistance (Pereira et al. 2015; Souza et al. 2019). These and other limitations inherent in traditional methods of management further illustrate the need to explore options for the biological control of *D. v. virgifera*.

Within their native habitat in Central America, natural enemies of the *D. v. virgifera* include parasitoid flies and wasps and entomopathogenic nematodes (Kuhlmann et al. 2005). In Nebraska, entomopathogenic nematodes from the genera *Heterorhabditis* and *Steinernema* were found in corn fields (Oliveira-Hofman 2018) and both have species capable of infecting rootworms (Toepfer et al. 2009). Arthropod predators of *D. v. virgifera* include predatory mites (Prischmann et al. 2011) and predatory insects within the orders of Coleoptera, Diptera and Hymenoptera (Toepfer et al. 2009). A study conducted in the same region of Nebraska as the current study evaluated the effectiveness of carabid beetles as biocontrol agents of *D. v. virgifera.* The results indicated that predation did not occur in the field, evidence that additional studies of other predatory arthropods are needed (Oliveira-Hofman et al 2020). Currently, there are very few

published studies evaluating the potential of spiders to be effective biological control agents of *D. v. virgifera* (Lundgren et al. 2009; Lundgren and Fergen 2011; Toepfer et al. 2009). Lycosids and linyphiids have been shown to prey on larvae while adult beetles have been preyed on by Agelenidae, Araneidae, Theridiidae and Thomisidae. The data from this study will expand on the current knowledge of spider predation of *D. v. virgifera* and potentially inform rootworm management recommendations in the future.

Western bean cutworm (*Striacosta albicosta* Smith) is a lepidopteran within the family Noctuidae that is a native to North America and an important pest of corn and dry beans (Smith et al. 2019). Historically, *S. albicosta* was first described in the 1880s in Arizona (Smith, 1887) and by the 1950s was found throughout the western Great Plains, including Nebraska (Hagan, 1962). Over the past two decades, *S. albicosta* has expanded its range eastward to include 22 additional states, the Canadian provinces of Ontario and Quebec, and further north into Nova Scotia (Smith et al. 2019). The larvae of *S. albicosta* feed on the reproductive tissues of corn plants, including tassels, silks and kernels (Smith et al. 2019). Significant yield losses of 15 bushels per acre can occur with only a single *S. albicosta* larva per ear (Paula-Moraes et al. 2013). Management of *S. albicosta* typically consists of foliar insecticide applications, primarily pyrethroids in Nebraska (Archibald et al. 2018), and transgenic corn expressing the Vip3A *Bt* protein (Unglesbee 2017) due to field-evolved resistance of transgenic corn expressing Cry1F (Smith et al. 2017; Coates et al. 2020). Unfortunately, there is currently evidence that suggests a reduction of pyrethroid efficacy against *S. albicosta* (Archibald et al. 2018); however, the nature and scope of this reduction is still in question (Montezano et al. 2019). Additionally, older instars of *S. albicosta* larvae have shown reduced susceptibility to the Vip3A protein in

laboratory bioassays (Farhan et al. 2019). The results of these studies illustrate the need for additional sustainable control methods against *S. albicosta*.

In addition to these more traditional management techniques, conservation biological control can be a useful tool in the control of *S. albicosta*. As a native insect of North America, the western bean cutworm has a number of natural enemies that prey upon eggs, larvae and adults including the insect predators Coccinellidae and Nabidae (Blickenstaff 1979) and the spiders Salticidae and Tetragnathidae (Archibald 2017). Currently, there are no published studies exploring the predation of *S. albicosta* by spiders in the field; however, spiders have been shown to feed on the eggs (Pfannenstiel 2008) and larvae (Clark et al. 1994) of other noctuid pests. This study will serve to fill in knowledge gaps concerning *S. albicosta* predation and the potential for biological control by spiders.

The ecology and behavior of *S. albicosta* and *D. v. virgifera* in corn dictates which guilds of spiders may interact with and prey upon them in the field. Early instar western bean cutworm larvae are exposed on the top half of the corn plant and therefore may encounter ambush predators in the crop canopy. Crab spiders (Araneae: Thomisidae) are ambush predators commonly collected in corn agroecosystems (Chapter 2) that sit on leaves or blossoms to wait for prey (Foelix 2011) and can perceive motion from up to 20 cm away (Homann 1934). These characteristics give thomisids the potential to locate and subdue lepidopteran larvae that come within their reach; therefore, Thomisidae were evaluated for western bean cutworm (*Striacosta albicosta*) DNA. Western corn rootworm larvae are located below the soil surface which may facilitate predation by ground

hunters. Wolf spiders (Araneae: Lycosidae) are cursorial ground-hunters that utilize movement vibrations and visual cues to find and capture prey (Foelix 2011).

Materials and Methods

Field Sites

Twelve field sites (8 in 2017 and 4 in 2018) were selected based on the expectation of having agronomic characteristics considered to be "conventional" (n = 6) or "conservation" (n = 6). Conventional fields were generally characterized by the planting of continuous corn, increased pest pressure leading to an increase in pesticide use and the use of disruptive management methods such as conventional tillage. Conservation fields were generally characterized by the use of crop rotation, non-crop habitat borders, reduced pesticide use and reduced or no-till practices.

Target prey availability surveys

The abundance of western bean cutworm and western corn rootworm within all field sites during both years was evaluated. During the month of July, field sites were scouted for *S. albicosta* egg masses, larvae and adults once per week. At four points along each transect, ten randomly selected corn plants 2 – 6m offset from the transect line were thoroughly searched for the presence of *S. albicosta*, producing data for 40 plants per field, per week. Through July and August, *D. v. virgifera* abundance was measured using sticky traps (Trece Inc. Pherocon AM No-Bait) placed in the field every week. The traps were installed just above ear-height on eight randomly selected plants per field on alternating sides of the transect 20m apart. Traps were then collected after one week and trapped *D. v. virgifera* adult beetles were counted.

Spider Collection

Spiders and target prey were collected from each field site using a variety of sampling techniques along set transects (Figure 2.1). In 2017, field work occurred from May 22 through August 22 and was completed over two days with four field sites visited each day. Night collections were conducted on June 14 -15 and July 26-27 after nightfall. In 2018, field work occurred from May 31 through August 30 and was completed in one day with all four field sites visited in the same day. Night collections were conducted on June 28 and August 2. For each collection date, the order in which field sites were visited was randomized to avoid sampling bias. Collected spiders were placed in microcentrifuge tubes or vials with 95% ethanol and transported back to the lab where they were stored at -20 °C. Spiders belonging to the families Lycosidae and Thomisidae were identified to lowest possible taxonomic level (Lycosidae: Dondale and Redner 1990; Thomisidae: Dondale and Redner 1978) and separated for future gut-content analysis.

Molecular Gut-Content Analysis

DNA Extractions

Samples were decontaminated prior to extraction using a three-step wash procedure modified from Curry et al. (2015) (K. Athey, personal communication). Each sample was removed from its storage vial and dipped for 15 seconds in each of three wash fluids in the following order: 5% bleach, deionized water and 95% ethanol. After decontamination, each sample was placed in a sterile and dry microcentrifuge tube for DNA extraction. Total body DNA extractions were conducted for all specimens except lycosids with a body size larger than 15 mm, in which case the opisthosoma only was used for extraction (Macias-Hernandez et al. 2018). The extractions were performed

using the DNEasy Blood and Tissue kit (Qiagen) by following the protocol provided by the manufacturer. Extracted DNA samples were stored at -20 °C until they could be analyzed through PCR.

Primers

Previously developed species-specific primers for both *S. albicosta* and *D. v. virgifera* were used for this study. These primers were the *S. albicosta* -specific primer pair F1-221 and R2-491 (Archibald 2017) and the *D. v. virgifera* -specific primer pair E-F364 and G-R358 (Peterson 2012; Oliveira-Hofman et al. 2020) (Table 3.1). These primers target the cytochrome c oxidase I (COI) gene. In addition to screening for non-targets and false negatives in the original description of these primers, additional vetting was conducted for this study. For the *S. albicosta*-specific primer pair test, five late instar *S. albicosta* larvae were cut into 4-5 pieces each and DNA was extracted. The resultant DNA samples were processed through PCR using the primer pair used in this study. The same evaluation was conducted for the *D. v. virgifera*-specific primer pair with seven adult *D. v. virgifera* used as the samples.

Table 3.1 Sequences of species-specific primers used in this study (**Tm**: melting temperature; **bp**: basepairs)

Primer Name	Direction	Primer Length (bp)	Tm (°C)	Sequence (5'-3')	Amplicon Length (bp)
WBC F1-221	Forward	25	57.6	TGGTAATTGATTAGTACCCCTAATG	270
WBC R2-491	Reverse	25	55.0	AAATAAAGGTATTTGATCAAATGAC	
WCR E-F364	Forward	19	53.3	CGGATGAACAGTTTACCCG	219
WCR G-R358	Reverse	23	53.0	ATAGAAGAAGGATTGCTGTAACG	

PCR & Gel Electrophoresis

Polymerase chain reaction (PCR) analysis was conducted using a T100™ thermal cycler (Bio-rad laboratories, Hercules, California). For *S. albicosta*, the PCR process was carried out with the following PCR protocol: initial denaturing step of 94 °C for 1 min,

followed by 40 cycles of 94 °C for 1 m (denaturation), 48 °C for 45 s (annealing), and 72 °C for 45 s (elongation), and a final extension step of 72 °C for 10 min (Archibald, 2017). Each 25 μL reaction mix consisted of: nuclease-free water (17.375 μL), Takara 10x Ex buffer (2.5 μL), Takara dNTP mixture (2 μL), primer pair (0.5 μL of each F1-221 and R2-491), Takara Taq (0.125 μL) and template DNA (2 μL). Reaction success was determined by electrophoresis of 10 μL of PCR product in 2% Fisher Agarose gel stained with GelRed (1 μL/10mL of TBE buffer). Electrophoresis was allowed to run for 40 minutes at 150 volts in 1x TBE buffer before visualization with a UV transilluminator (Spectroline Select Series model# TC-312E).

For *D. v. virgifera*, the PCR process was carried out with the following PCR protocol: initial denaturing step of 94 °C for 15 min, followed by 50 cycles of 94 °C for 15 s (denaturation), 56 °C for 30 s (annealing), and 72 °C for 30 s (elongation), and a final extension step of 72 °C for 10 min (Peterson 2012; Oliveira Hofman et al. 2020). Each 25 μL reaction mix consisted of: nuclease-free water (17.375 μL), Takara 10x Ex buffer (2.5 μL), Takara dNTP mixture (2 μL), primer pair (0.5 μL of each E-F364 and G-R358), Takara Taq (0.125 μL) and template DNA (2 μL). Reaction success was determined by electrophoresis of 10 μL of PCR product in 2% Fisher Agarose gel stained with GelRed (1 μL/10mL of TBE buffer). Electrophoresis was allowed to run for 40 minutes at 150 volts in 1x TBE buffer before visualization with a UV transilluminator (Spectroline Select Series model# TC-312E).

Testing DNA Detectability Half-Life

Spider Collection

Adult and immature spiders within the families Lycosidae and Thomisidae were collected live from corn fields near the West Central Research and Extension Center in North Platte, Nebraska (41.086866, -100.778005) and brought back to the lab.

Spider Laboratory Housing

Housing of spiders in the laboratory generally followed methods described in Rundus et al. (2011). All lycosids and adult thomisid spiders were kept in small (58mm x 58mm x 79mm) rectangular plastic containers with lids (AMAC 760C Flat Top Box, Crystal). Each plastic container was covered on the exterior with masking tape to eliminate between-container spider interaction or stress. Two of the interior walls of each container were lined with nylon mesh screen to allow the spiders to climb. Each container had a hole (1cm diameter) drilled in the top of the lid with a rubber stopper (Rudimen; size #000) placed in the hole (for easy feeding access). A second hole (1cm diameter) was drilled in the bottom of each container with a dental wick (Dynarex; N/S #2 medium) fed through the hole to provide the spiders with constant moisture. The containers (up to 35) were placed within rectangular plastic bins (Sterilite ID Box; 50.5 cm x 39.1 cm x 16.5 cm) with a cutting board (Mainstays 15x20 Poly Cutting Board; 5 cm) from the bottom and water filling the bottom of the plastic bin. A total of 35 holes (1.3 cm diameter) were drilled into the cutting boards to allow for the dental wicks. The dental wicks from each container were fed through the holes in the cutting board and into the water, thus providing a continuous source of moisture. The bins were placed in

incubators (Percival E36HO Plant Growth Chamber) set at 26.6 °C, 70-80% relative humidity and 16:8 h (L:D) cycle.

Immature thomisids were kept in small plastic portion containers (Dart Solo Cups 2oz.) with 2 cm of plaster of Paris at the bottom to facilitate moisture retention and therefore reduce the risk of desiccation (Schmidt et al. 2013). Lids with several small holes for ventilation were placed onto each container and the containers were placed in one layer onto cafeteria trays. The trays were placed in incubators (Percival E36HO Plant Growth Chamber) set at 26.6 °C, 70-80% relative humidity and 16:8 h (L:D) cycle.

Feeding Trials

Once spiders were collected and set up in the lab, they were fed a baseline meal of one cricket (pinhead to 0.6 cm long) for lycosids and one wingless *Drosophila* for thomisids and then starved for 7 days with only water being provided. After the starvation period, spiders were fed one 3rd instar *D. v. virgifera* larva for lycosids or one neonate *S. albicosta* larva for thomisids and then randomly assigned to one of nine time intervals: 0, 2, 4, 8, 16, 24, 48, 72, or 96 hours. Spiders were observed every 5 minutes to check for feeding and were allowed the opportunity to feed for up to 120 minutes. Any spiders that did not feed within that time frame were removed from the study and released. Chaser prey was not used in this study due to evidence suggesting chaser prey does not have a significant effect on prey DNA detectability (Fülöp et al. 2019). After feeding on the target prey item, each spider was placed in chilled 95% ethanol and stored at -20 °C once the assigned time interval had passed. A minimum of 10 spiders from each family and for each target prey type were randomly assigned to each time interval.

Data Analysis

DNA Detectability Half-Life

To determine the rate of prey DNA decay in the digestive tract of the predator and the half-life for detectability, feeding trial data were analyzed by fitting a four-parameter logistic curve (dose response) regression equation to the proportion positive at each time period using SigmaPlot (v. 14.5; Systat Software Inc., San Jose, California, USA) (after Greenstone et al., 2007; Payton et al., 2003; Peterson et al. 2018).

Results

Target Pest Abundance Results

Striacosta albicosta

During the 2017 collecting period from July 2 through July 25 and across all field sites, a total of 32 western bean cutworm egg masses were counted through scouting. The rate of infestation in 2017 peaked on July 18, with field site TB exhibiting a 20% infestation rate (Figure 3.1). In 2018, from July 5 through August 2, a total of 19 egg masses were counted through scouting. The 2018 infestation rate peaked on July 25, with a 13% infestation rate at field site SA18 (Figure 3.2).

Figure 3.1 2017 seasonal infestation of *S. albicosta* at each field site. Infestation rate based on number of plants out of 40 per field infested with egg masses, larvae or adults. Pie charts indicate the relative proportion of each life stage observed (EM = egg masses; L = larvae: Ad = Adults). Data points without pie charts represent 100% egg masses observed.

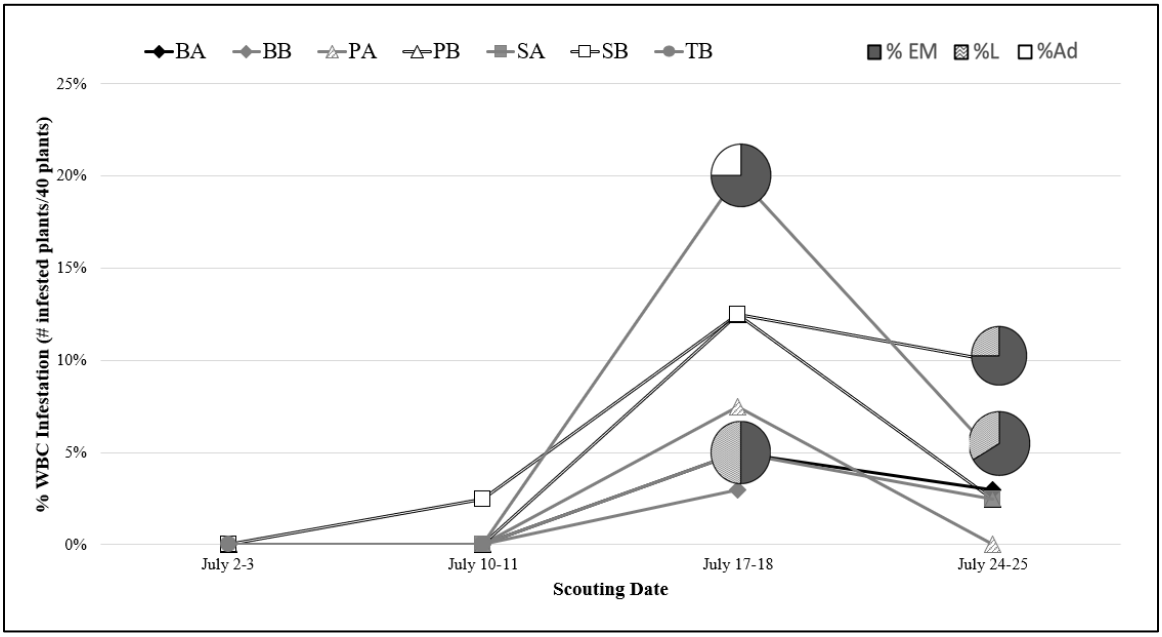
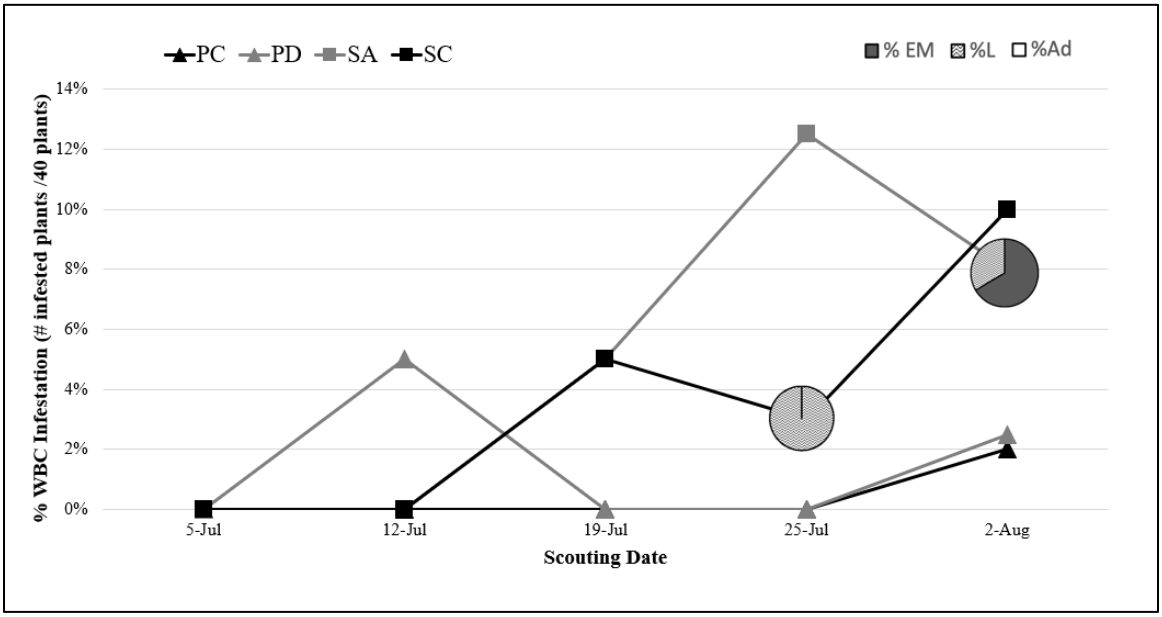


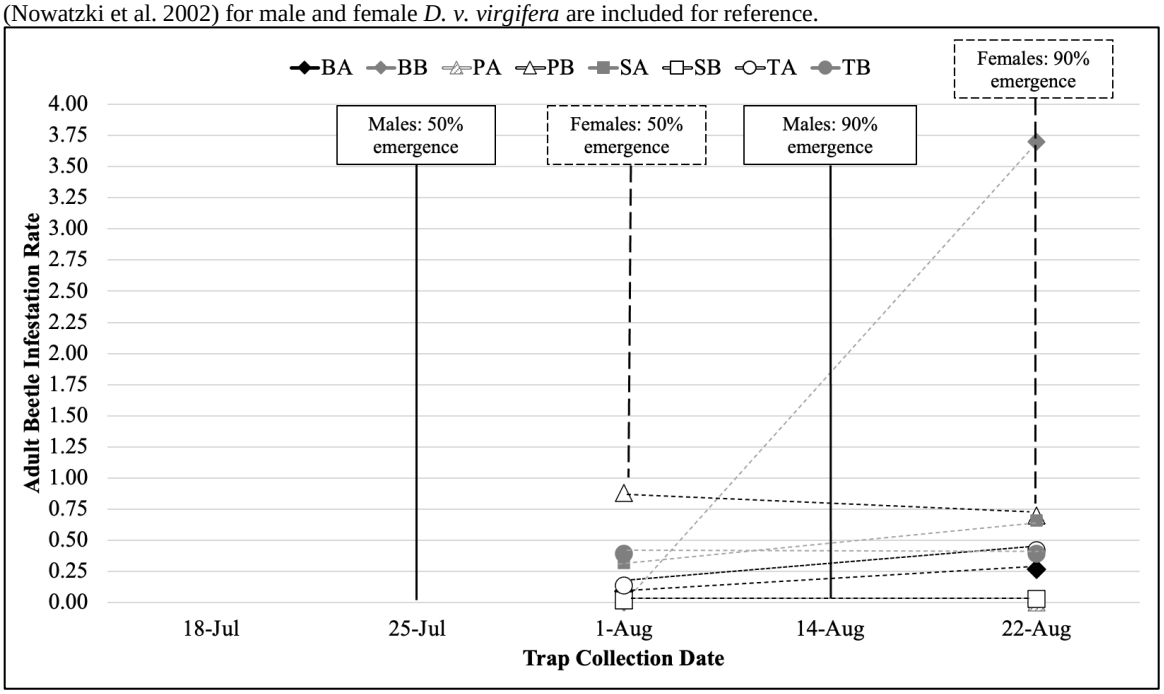
Figure 3.2 2018 seasonal infestation of *S. albicosta* at each field site. Infestation rate based on number of plants out of 40 per field infested with egg masses, larvae or adults. Pie charts indicate the relative proportion of each life stage observed (EM = egg masses; L = larvae: Ad = Adults). Data points without pie charts represent 100% egg masses observed.



Diabrotica virgifera virgifera

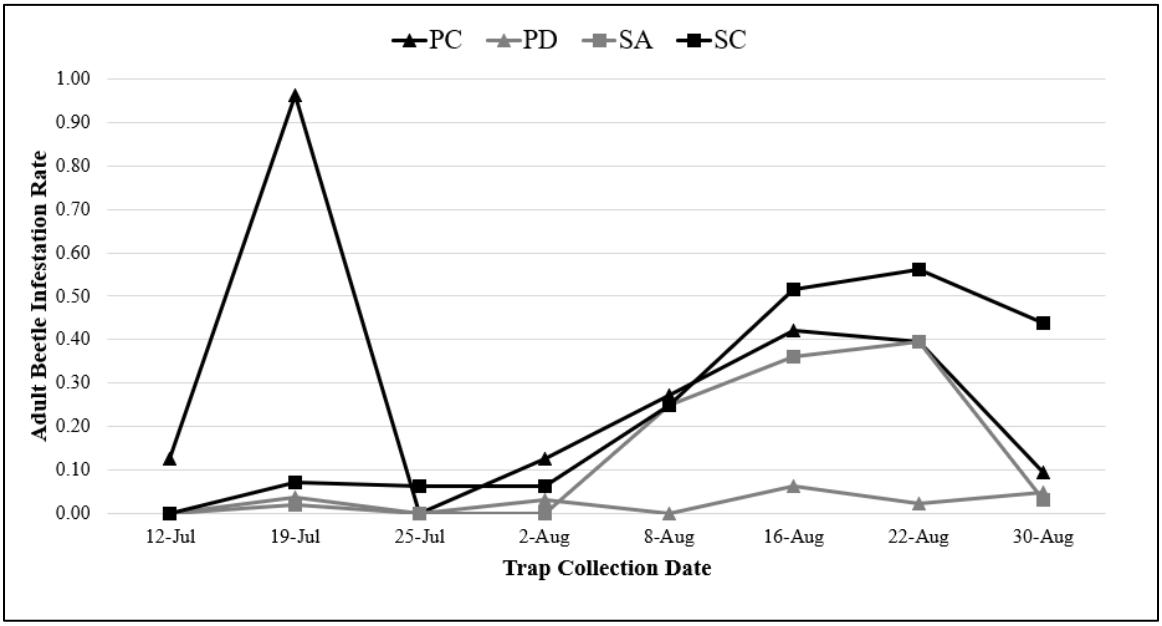
In 2017 a total of 482 *Diabrotica virgifera virgifera* beetles were counted from sticky card traps between July 10 and August 22; however, samples from July 18, July 25 and August 14 were lost. Adult *D. v. virgifera* infestation peaked on August 22, 2017 with a total of 3.70 beetles per trap per day at field site BB (Figure 3.3). To inform gaps where sticky card trap data were lost, degree day calculations were conducted using formulas from Allen (1976) and air temperature data from weather stations through Mesonet (Shulski et al. 2018). Emergence benchmarks for male and female *D. v. virgifera* from (Nowatzki et al. 2002) were included to illustrate adult beetle emergence throughout the season.

Figure 3.3 2017 seasonal infestation rate of *D. v. virgifera* at each field site. Data were recorded for August 1 and August 22 only due to a loss of traps for July 18, July 25 and August 14. Emergence benchmarks calculated from (Nowatzki et al. 2002) for male and female *D. v. virgifera* are included for reference.



In 2018 a total of 314 beetles were counted from sticky card traps from July 12 through August 30. Adult *D. v. virgifera* infestation rate peaked on July 19, 2018 with 0.96 beetles per trap, per day at field site PC (Figure 3.4).

Figure 3.4 2018 seasonal infestation rate of *D. v. virgifera* at each field site. Infestation rate determined by average number of beetles per trap per day at each field site.



Molecular gut-content analysis of thomisids for *S. albicosta*

Field Collected Thomisidae

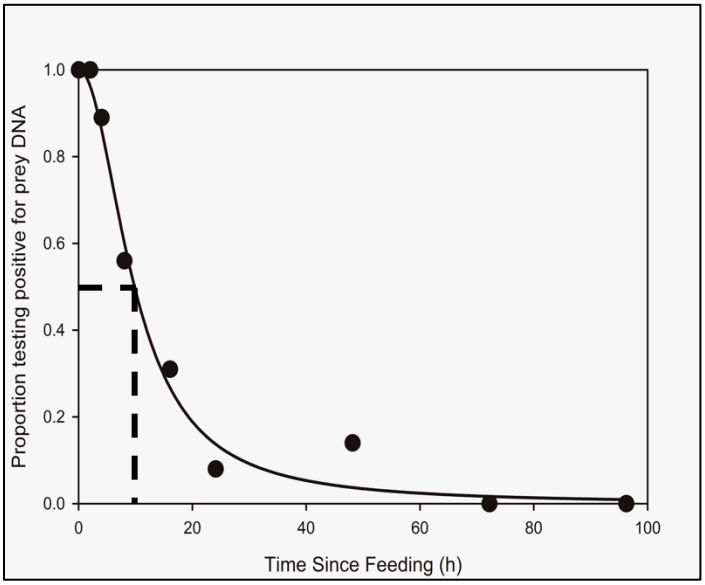
A total of 36 thomisids were collected from the field- immatures (n = 19), *Mecaphesa celer* (n = 1), *Xysticus acquiescens* (n = 1), *Xysticus ferox* (n = 14) and *Xysticus gosiutus*(n = 1). Of these specimens, 25 were evaluated by molecular gut-content analysis, with zero testing positive for *S. albicosta* DNA.

Feeding trial Thomisidae

A total of 117 thomisids were collected June – August 2019 and returned to the lab where they were kept for feeding trials. Of the 117 collected specimens, 29 died before completing the trial and 3 did not feed on the target prey and were released. The remaining 85 thomisids were comprised of 74% immatures, 20% from the genus *Mecaphesa*, 1% from the genus *Misumenoides* and 1% from the genus *Xysticus.* Feeding trials indicated that *S. albicosta* neonate larvae are a readily accepted source of prey for

thomisids in the laboratory. Overall, 88 thomisids were offered one neonate *S. albicosta* larva and 85 (97%) of the spiders captured and fed on the larva. The remaining 3 spiders did not feed. Analysis of the thomisid-*S. albicosta* feeding trial specimens yielded a nonlinear regression decay curve ($r^2 = 0.98$, $F_{1,8} = 507.99$, $P = <0.0001$) with a DNA detectability half-life of 9.77 h (Fig. 3.5).

Figure 3.5 Non-linear regression decay curve of *S. albicosta* DNA in tested thomisids. A detectability half-life of 9.77 h was determined



Molecular gut-content analysis of lycosids for *D. v. virgifera*

Field Collected Lycosidae

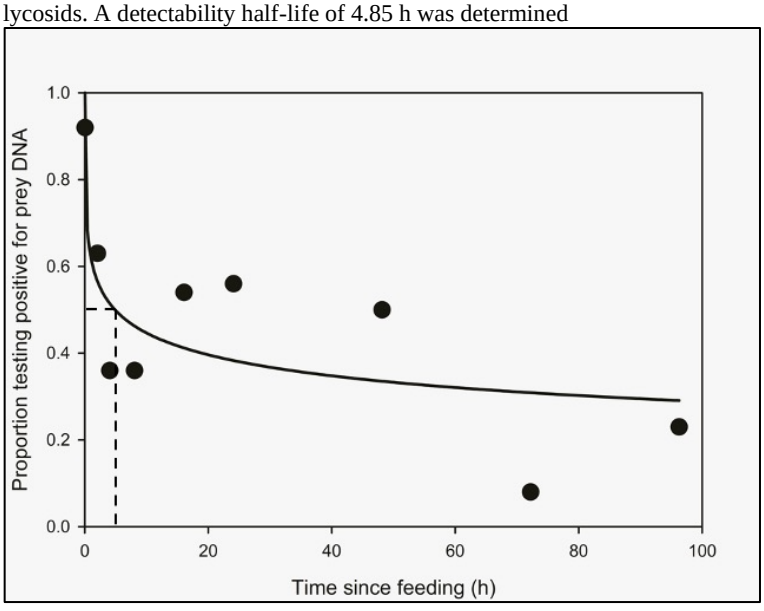
A total of 605 lycosids were collected from the field composed primarily of *Schizocosa ocreata* (n = 262), immatures (n = 208) and *Schizocosa avida* (n = 52). Of these, 526 lycosids were evaluated by molecular gut-content analysis, with 2 individuals testing positive for *D. v. virgifera* DNA which is a positivity rate of less than 1%. Both positive specimens were collected from field site SA, but in different years. The specimen collected in 2017 was captured by pitfall trap on June 19 and was a male

Schizocosa ocreata. In 2018, the positive specimen was hand collected on August 22 and was an immature *Tigrosa* sp.

Feeding trial Lycosidae

A total of 192 lycosids were collected April – June 2019 and returned to the lab where they were kept for feeding trials. Of the 192 collected specimens, 36 died before completing the trial and 47 did not feed on the target prey and were released. The remaining 109 lycosids were comprised of the following taxa: 45% *Schizocosa*, 31% immature, 11% *Pardosa*, 8% *Trochosa* and 2% *Varacosa.* Overall, 156 lycosids were offered a single third instar *D. v. virgifera* larva and 109 (70%) fed on the offered larva while 47 (30%) did not feed. Analysis of the lycosid-*D. v. virgifera* feeding trial specimens yielded a nonlinear regression decay curve ($r^2 = 0.58$, $F_{1,8} = 11.92$, P=0.01) with a DNA detectability half-life of 4.85 h (Fig. 3.6).

Figure 3.6 Non-linear regression decay curve of *D. v. virgifera* DNA in tested lycosids. A detectability half-life of 4.85 h was determined



Discussion

This study sought to determine whether Thomisidae and Lycosidae prey upon *Striacosta albicosta* and *Diabrotica virgifera virgifera*, respectively. Spider sampling and target prey availability surveys revealed both spider families and target prey taxa are concurrently present in the sampled fields. *D. v. virgifera* exceeded the economic threshold level of 2.0 beetles per trap per day (Seiter 2018) at field site BB on August 22, 2017 with an infestation rate of 3.70 beetles per trap per day. *S. albicosta* met or exceeded the recommended economic threshold for Nebraska of 5-8% of scouted plants infested with eggs or larvae (Paula-Moraes et al. 2013) during both years. This occurred in 2017 between July 17 and July 25 while in 2018, the economic threshold was met or exceeded between July 12 and August 2.

In the current study, none of the thomisids screened for *S. albicosta* DNA tested positive. This can be partly explained by the point in the season the spiders were collected in relation to *S. albicosta* presence in the fields. In 2017, only 35% (n = 7) of thomisids were collected during the period when *S. albicosta* was present while in 2018 20% (n = 1) of thomisids were collected when *S. albicosta* was present. Despite this, during laboratory feeding trials, *S. albicosta* larvae were readily consumed by thomisids which is similar to the results evaluating thomisid predation of another species of noctuid moth (Pearce et al. 2004). Additionally, thomisids present in soybean fields showed preference for defoliating lepidoptera larvae over other prey during field cage experiments (González et al. 2009). However, contrasting results were found in a laboratory prey choice (Pérez-Guerrero et al. 2013) experiment in which thomisids showed preference for *Drosophila* over Noctuidae larvae. The inconsistency of thomisid

predation of noctuid larvae is most likely a result of environment and prey availability. While spiders are polyphagous predators, they generally select prey based on nutritional needs and avoidance of toxic prey (Toft 1999). Additionally, there is some evidence that spiders exhibit food imprinting, in which early feeding experience affects prey choice later in the life of the spider (Punzo 2002). These characteristics, the results of this study and the limited amount of literature focused on thomisids as potential biological control agents illustrate the need for additional and perhaps more targeted experiments.

In this study, two lycosids (*Schizocosa ocreata* and *Tigrosa* sp.) of the 526 screened for *D. v. virgifera* DNA tested positive which is a positivity rate of less than 1%. This is lower than the positivity rate of 6% for *Pardosa* sp. (n = 14 tested) and 100% for *Schizocosa* sp. (n = 2 tested) determined by Lundgren and Fergen (2011); however, the current study relied on natural infestations of *D. v. virgifera* while Lundgren and Fergen (2011) artificially infested field sites with eggs. It is also important to note that, while lycosids were the most abundant spider taxon in the current study, the trophic relationships between fluid-feeding predators (e.g., spiders) and *D. v. virgifera* are not significantly affected by the predator's abundance, diversity or evenness (Lundgren and Fergen 2014). *D. v. virgifera* larvae also possess hemolymph defenses that provide protection from entomopathogenic nematodes (Robert et al. 2017) as well as some predators (Welch and Lundgren 2014). These defenses make *D. v. virgifera* larvae a suboptimal prey choice for predators, including spiders, which may explain the minimal level of *D. v. virgifera* predation by lycosids in the field.

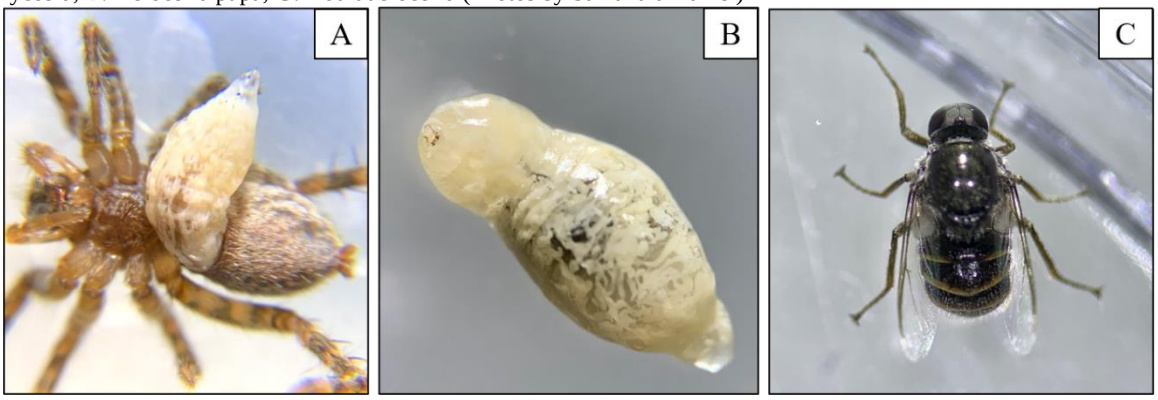
The primary purpose of feeding trials conducted in this study was to determine the detectability half-life of target prey DNA in the guts of spiders. After 9.77 hours, 50%

of screened thomisids tested positive for *S. albicosta* DNA while *D. v. virgifera* DNA was detectable in 50% of screened lycosids after 4.85 hours. These results are similar other studies involving aphid DNA detectability in lycosids (Kuusk et al. 2008) and stink bug DNA detectability in oxyopids (lynx spiders) (Athey et al. 2017). There are examples, however, of significantly longer DNA detection windows in spiders including a 78-hour half-life for Mediterranean fruit fly DNA in lycosids (Monzo et al. 2010) as well as a 5-day half-life of leafhopper DNA in philodromids (running crab spiders) (Fülöp et al 2019). The determination of decay rates is necessary when using molecular gut-content analysis results to draw conclusions about the impact of trophic interactions (Greenstone et al. 2014). Shorter detectability half-lives mean there is a smaller window of detection for field collected predators and therefore in-field predation may be underestimated. It is also necessary to consider feeding frequencies of predators in the field. There is evidence that both lycosids and thomisids exhibit low feeding frequencies with as little as one prey item consumed per day (Nyffeler and Breene 1990). When this is considered concurrently with lycosid and thomisid short detectability half-lives of 4.85 hrs and 9.77 hrs respectively, the chances of collecting a predator that recently fed on target prey are reduced. These results illustrate the variability of prey DNA detectability over time, the importance of DNA half-lives in understanding the impact of trophic relationships and the need for additional research concerning spider predation of agronomic pests.

Some important observations were made during the laboratory feeding trials of this study regarding spider mortality and the ability of lycosids and thomisids to successfully subdue and prey upon adult *D. v. virgifera* beetles. While mortality of

feeding trial specimens was primarily a result of desiccation or unknown factors like disease, two specimens (one thomisid and one lycosid) were parasitized by a small headed fly of the family Acroceridae (Figure 3.7). These parasitoids are the only known endoparasitic Diptera that are host-restricted to and have coevolved with spiders (Schlinger 1987).

Figure 3.7 Endoparasitic Diptera of the family Acroceridae. **A**: Final instar larva emerging from immature lycosid; **B**: Acrocerid pupa; **C:** Adult acrocerid (Photos by Samantha Daniel)

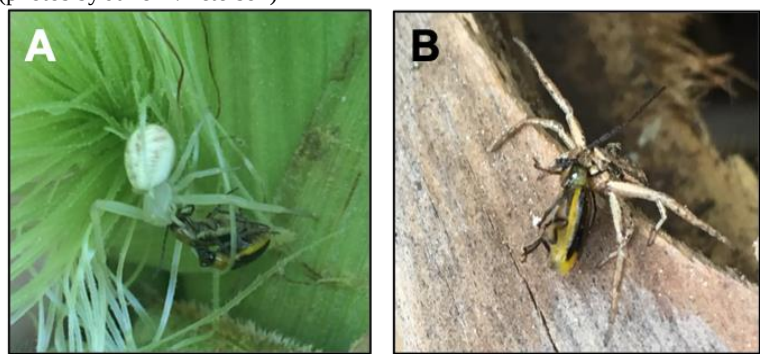


With a wingspan of roughly 3.8 cm (Peairs 2002), adult *S. albicosta* are fairly large and strong flying moths and were therefore not considered in this study as potential prey for spiders in corn. Because *D. v. virgifera* utilize chemical defenses (Pasteels et al. 1994) they may pose a challenge to spiders utilizing them as prey. For this reason, adult *D. v. virgifera* are unlikely to serve as preferred prey for most spiders. The two lycosid specimens testing positive for *D. v. virgifera* DNA in this study likely fed on different developmental stages of this insect. The positive specimen collected June 19, 2017 probably fed on a larva while the 2018 specimen collected August 22 may have fed on eggs, an adult or possibly scavenged (Vidal et al. 2004).

In the earlier stages of this project, preliminary feeding trials were conducted with thomisids and lycosids to evaluate predation of adult *D. v. virgifera* beetles. Predation of *D. v. virgifera* adults by thomisids has been observed in the field (Figure 3.8). Despite

this, only seven thomisids were able to subdue and consume an adult beetle in the laboratory out of over 50 tested. Field observations of predation may have involved sick or dying beetles, or perhaps the thomisids were adapted specifically to an agroecosystem food chain.

Figure 3.8 Thomisidae preying upon western corn rootworm beetles (photos by Julie A. Peterson)



Lycosids were also considered potential predators of adult beetles due to their epigeal behavior and the emergence of the adult beetles from the soil as well as the oviposition of eggs into the soil by female beetles. Preliminary feeding trials indicated an overall inability of lycosids to subdue *D. v. virgifera* adults. These observations may be explained by the fact that adult *D. v. virgifera* utilize chemical defenses that protect them from predation (Pasteels et al. 1994).

The results of this study indicate that some predation of *D. v. virgifera* by Lycosidae is occurring in the field. The short detectability half-life of *D. v. virgifera* DNA in lycosids and the propensity of these spiders to feed infrequently means that predation of this pest may be occurring more often than the results indicate. However, it is important to consider the suitability of chrysomelid beetles as prey for spiders. The ability of larvae to sequester toxins makes them a less-favorable food-source than other insects. While none of the thomisids screened in this study tested positive for *S. albicosta*

DNA, the small thomisid sample size and the readiness of these spiders to feed on *S. albicosta* larvae during laboratory feeding trials warrants further investigation into this predatory group's impact on *S. albicosta* in the field.

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CHAPTER 4: Project Summary and Conclusions

This project sought to determine the composition of spider communities in western Nebraska agroecosystems and the impact of agronomic practices on those communities, as well as the trophic relationship between spiders and two key pests of corn and the potential for biological control of these pests by spiders.

The first objective of this project was to describe the diversity and abundance of spider communities in western Nebraska corn agroecosystems under conservation and conventional management. The results show fairly diverse and abundant spider communities within Nebraska corn fields that exhibit seasonal and field site differences in abundance and composition. Additionally, the effects of crop rotation and strip-tillage did not significantly impact these communities; however, insecticide use did. These data can help to inform future biological control programs. Currently published literature describing the spider species within Nebraska do not list 22 of the species found in this study. The addition of these species to the Nebraska record will facilitate an increased understanding of Nebraska arthropods and expand on what is known about the range of each species.

The second objective was to determine the strength of the trophic relationship between spiders and two key pests of corn: *Striacosta albicosta* (Smith) and *Diabrotica virgifera virgifera* (LeConte). Based on the spider community results from Chapter 2, an understanding of spider and pest ecology and behavior and the prey availability data collected, two key spider families were selected as potential predators of the target pest species. The crab spiders (Thomisidae) were selected as potential predators of *S. albicosta* larvae and wolf spiders (Lycosidae) were considered potential predators of *D. v.*

virgifera eggs, larvae or adults. Field collected spiders were screened for target prey DNA: none of the screened thomisids tested positive for *S. albicosta* DNA while only two lycosids tested positive for *D. v. virgifera* DNA. While these results indicate no to very low predation of these pests in the field, a variety of factors including short DNA detectability windows, spider feeding habits and prey availability at the time of spider capture may help to describe these results and illustrate a need for additional studies.

Studies investigating the potential biocontrol services of spiders are numerous; however, the focus has primarily been on only a few families (Uiterwaal and DeLong 2020). This is most likely a result of the most abundant or most commonly captured spider families in agroecosystems being given priority in trophic interaction studies. More targeted or selective sampling procedures may reveal an increased abundance in more elusive spider families. The biocontrol potential of spiders is an important and potentially consequential area of study that is also quite complex. A recent review of over 50 studies conducted in grape, cabbage, wheat and rice agroecosystems found that spiders suppressed pest populations in 79% of cases (Michalko et al. 2019). In 62.5% of studies, spiders enhanced crop performance while in 37.5% of studies crop performance was reduced by spiders (Michalko et al. 2019). This review also found that active hunter spider diversity enhanced pest suppression significantly more than the diversity of web-builders (Michalko et al. 2019). In addition to direct predation, spiders are also capable of exerting non-consumptive effects on pests that can reduce crop damage as well as disease transmission from insect vectors (Michalko et al. 2019; Tholt et al. 2018). Future studies in spider biological control potential should consider these factors while also taking advantage of advances in molecular quantification of predator diets.

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APPENDIX A: Field Site Data

Table 2.7 Agronomic and field history data collected from farmers via survey.

Type	Field	Field ID	GPS Coordinates	Crop Variety	Irrigated	Crop Rotation	Tillage	Insecticide Use	Herbicide Use	Fertilizer Use	Cover Crops	Non-Crop Borders
Conservation	Paulman A	PA	41°03'17.5"N 101°06'10.7"W	Not specified	Yes (detailed info not avail)	Yes (2014 wheat; 2015 corn; 2016 corn)	No	At plant	Yes	Yes	Yes	None
	Paulman B	PB	41°02'30.8"N 101°14'53.1"W	Not specified	Yes (detailed info not avail)	Yes (2014 beans; 2015 popcorn; 2016 corn)	No	At plant	Yes	Yes	Yes	None
	Tucker A	TA	40°47'48.9"N 101°57'19.9"W	Non-GMO Viking 95 day corn	No	Yes (2014: millet; 2015: peas; 2016: wheat)	No	None	**5/12**: Roundup (24oz), Atrazine (1lb), Balance Flex (3oz)	**5/25**: 32-0 16g; 10-34-0 5g	None	Brome grass ditches
	Tucker B	TB	40°43'53.0"N 101°57'16.5"W	Dekalb 5084 & Pioneer 9998	No	Yes (2014: corn; 2015: yellow peas; 2016: wheat)	No	None	**6/14:** Roundup (40oz)	**5/6**: 32-0-0 22gpa, 10-31-0-0.5 5gpa	None	None
Conventional	Barnhill A	BA	41°05'26.1"N 101°30'53.5"W	Mycogen 10 Z28 SmartStax	Yes (detailed info not avail)	Yes (2014 Soy; 2015 corn; 2016 soy)	Strip-till (**4/10**)	Seed trt (Mycogen 10Z28)	**4/22**: Atrazine (1lb), Lumax (48oz) **6/20**: Status (4oz), Halex (2qts)	**3/25:** N 150lbs, Phos. 50lbs; **5/10**: 9-27-0-3-0.5 10gal, 32-0-0 5gal; **7/1**: 32-0-0 10gal	None	Windbreak of trees to south

	Barnhill B	BB	41°06'59.9"N 101°39'03.2"W	Channel 209-53 STX RIB	Yes (detailed info not avail)	No (Corn since 2014)	Strip-till (4/7)	Seed trt: Channel 209-53 STX RIB; At plant: Counter 5.5lb/acre	**4/22**: Fultime (?)(48oz)	**3/25:** N 150lbs, Phos. 50lbs; **5/12**: 9-27-0-3-0.5 10gal, 32-0-0 5gal; **7/1**: 32-0-0 10gal	None	Interstate 80 along north side; south platte river 0.83km to north
	Spurgin A	SA17	41°05'29.4"N 101°23'26.3"W	Mycogen 767	Yes (11 acre in)	No (5 year corn)	Strip-till	Seed trt: Exceleron	**4/25**: Acuron (1.25qt), Whitematch (1pt)	N (271); Phos. (70); Potash (8); Sulfur (15); Zinc (.8)	None	Feedlot bordering north side
	Spurgin B	SB	41°04'18.2"N 101°27'37.6"W	5654 Bk	Yes (10 acre in)	No (5 year corn)	Strip-till	Seed trt: Exceleron	**4/20**: Acuron (1.25qt), Whitematch (1pt)	N (252); Phos. (70); Potash (8); Sulfur (15); Zinc (.8)	None	None
Conservation	Paulman C	PC	41°08'21.8"N 101°10'48.0"W	Not specified	Yes (detailed info not avail)	No	Strip-till	At plant	Yes	Yes	None	Interstate 80 along south side; small ponds, trees and S. platte river to north
	Paulman D	PD	41°08'33.3"N 101°10'16.3"W	Not specified	Yes (detailed info not avail)	No	Strip-till	At plant	Yes	Yes	None	Interstate 80 along south side; trees and S. platte river to north

Conventional	Spurgin A	SA18	41°05'29.4"N 101°23'26.3"W	DKC 56-45	Yes (detailed info not avail)	No	No	None	**5/10**: Acuron (1.25qt), Widematch (1pt), Powermax (28oz); **5/23**: Acuron (1.25qt), Powermax (28oz), Status (3oz)	**4/10**: 20-16-0.3 22gal; **4/26**: 8-20-5-5-0.5 14gal; **5/30**: 29-0-0.4 30gal; **7/10**: 32-0-0 29gal	None	Feedlot bordering north side
	Spurgin C	SC	41°05'27.6"N 101°24'32.9"W	C210-Z6	Yes (detailed info not avail)	No (Corn since 2015)	Strip-till (**4/14**)	None	**5/15**: Acuron (1.25qt), Widematch (1pt), Powermax (28oz); **5/28**: Acuron (1.25 qt), Powermax (28oz), Status (30oz)	**4/14**: 20-16-0.3 22gal; **4/28**: 8-20-5-5-0.5 14gal; **6/3**: 29-0-0-4 30gal; **7/20**: 32-0-0 30gal	None	None